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Docket Number **UCSD1690-1**

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(s)/APPLICANT(s)		
Given Name (first and middle [if any])	Family Name or Surname	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
Tor Luedtke	Yitzhak Nathan	San Diego, California La Jolla, California

Γ Additional inventors are being named on page ____ attached hereto.

TITLE OF INVENTION (280 characters max)

COMPOSITIONS AND METHODS FOR USE OF GUANIDINIUM DERIVATIVES OF GLYCOSIDES FOR IMPROVED CELLULAR TRANSPORT

CORRESPONDENCE ADDRESS

LISA A. HAILE
GRAY CARY WARE & FREIDENRICH
4365 Executive Drive, Suite 1100, San Diego, California 92121-2133
Phone: (858) 677-1456 Fax: (858) 677-1465

ENCLOSED APPLICATION PARTS (*check all that apply*)

X	Specification	*Number of Pages*	35		X	Entitled to Small Entity Status	Yes
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Respectfully submitted,

SIGNATURE Lisa Haile DATE September 12, 2003

Lisa A. Haile, Ph.D.
TYPED or PRINTED NAME REG. NO. 38,347

TELEPHONE (858) 677-1456

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PROVISIONAL

APPLICATION

for

UNITED STATES LETTERS PATENT

on

COMPOSITIONS AND METHODS FOR USE OF GUANIDINIUM DERIVATIVES OF GLYCOSIDES FOR IMPROVED CELLULAR TRANSPORT

by

Yitzhak Tor
Nathan Luedtke

Docket No.: UCSD1690-1
Drawings: 8 Sheets

Lisa A. Haile, Ph.D.
Gray Cary Ware & Freidenrich LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133

COMPOSITIONS AND METHODS FOR USE OF GUANIDINIUM DERIVATIVES OF GLYCOSIDES FOR IMPROVED CELLULAR TRANSPORT

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0001] The invention relates generally to bioavailability and delivery of therapeutic molecules and, more specifically, to guandinylated cyclic acetals and conjugation of such modified acetals to therapeutic compounds to increase the cellular uptake of such compounds, to include methods of preparing, therapeutic modalities using and products comprising such modified acetals.

BACKGROUND INFORMATION

[0002] Charged molecules over 500 amu typically exhibit poor bioavailability. This limits the delivery of many therapeutically active molecules to their intended targets. Polycationic molecules provide important exceptions to this generalization. Modification of Bovine Serum Albumin (BSA) with ethylene diamine produces "cationionized BSA", a highly effective antigen carrier. Despite its size (over 66,000 amu), cationized BSA efficiently enters cells via an unknown path involving adsorptive uptake. More recently, a number of poly-arginine peptides, peptoids, and peptidomimetics, have been found to exhibit highly efficient uptake into a wide range of mammalian cell types. The conjugation of such poly-Arg peptides to large molecules can facilitate the transduction of peptide, protein, and nucleic acid, conjugates into cells. The mechanism responsible for poly-Arg mediated transport is still unclear, but may involve a receptor mediated, non-endocytotic route.

[0003] Thus, an opportunity exists for exploiting such a poly-arginine peptide-like transduction mechanism for efficient uptake of therapeutically active molecules by eukaryotic cells.

[0004] In the present invention, guanidine-modified cyclic acetals, such as guanidinoglycosides, have been found to exhibit efficient uptake by eukaryotic cells in general as well as acting as effective cellular uptake enhancers when conjugated to other compounds. The

present invention provides such guanidine-containing modified natural/synthetic products that facilitate the efficient cellular transport of cargo molecules via covalent conjugation.

SUMMARY OF THE INVENTION

[0005] The present invention relates to compounds covalently conjugated to guanidinoglycosides that exhibit highly efficient uptake by eukaryotic cell cultures via a mechanism similar to cationionized peptides. In a related aspect, such conjugated products share the same uptake mechanism as the TAT and other arginine-containing peptides.

[0006] In one embodiment, a method of increasing the cellular uptake of a compound is provided which includes conjugation of a compound with a molecule having a guanidinylated cyclic acetal. In one aspect, such an acetal may be polymeric or non-polymeric. In a related aspect, a method of making such conjugates is also envisaged.

[0007] In another related aspect, the modified cyclic acetal is a natural or synthetic glycoside and more particularly, but not limited to, aminoglycosides, cardiac glycosides, disaccharides or other polysaccharides . Further, primary or secondary alcohol or primary or secondary amines of these glycosides are reacted with guanidinylating reagents to produce guanidinoglycosides. In one embodiment, such guanidinoglycosides are covalently bonded to molecules of interest, such as, but not limited to, therapeutically active molecules.

[0008] In a related aspect, guanidinylated cyclic acetal containing compounds include, but are not limited to, amikacin, gentamicin, kanamycin, neomycin, netilmicin, O-2,6-Diamino-2,6-dideoxy-beta-L-idopyranosyl-(1 to 3)-O-beta-D-ribofuranosyl-(1 to 5)-O-[2-amino-2-deoxy-alpha-D-glucopyranosyl-(1 to 4)]-2-deoxystreptamine, streptomycin, tobramycin, ouabain, deslanoside, digoxin, digitoxin, lantoside, gitoxigenin, bufalin and strophanthin.

[0009] Further, compounds covalently conjugated to the guanidinoglycosides of the present invention may include, but are not limited to, nucleic acids, nucleosides, proteins, peptides, amino acid residues, lipids, carbohydrates, synthetic organic compounds, metals, vitamins, small molecules, dyes, isotopes, antibodies, toxins ligands or any other compound that may need transport into a cell.

[00010] In one embodiment, the conjugates include, but are not limited to a nucleoside, where the nucleoside is a reverse transcriptase inhibitor (RTI). In a related aspect, such nucleosides

may include, but are not limited to, 3'-azido-3'-deoxythymidine, 2',3'-dideoxyinosine and 2',3'-dideoxycytidine. In another related aspect, the reverse transcriptase inhibitor is conjugated to a guanidine-modified aminoglycoside, such as guanidino-amikacin, guanidino-gentamicin, guanidino-kanamycin, guanidino-neomycin, guanidino-netilmicin, guanidino-O-2,6-Diamino-2,6-dideoxy-beta-L-idopyranosyl-(1 to 3)-O-beta-D-ribofuranosyl-(1 to 5)-O-[2-amino-2-deoxy-alpha-D-glucopyranosyl-(1 to 4)]-2-deoxystreptamine, guanidino-streptomycin and guanidino-tobramycin.

[00011] The present invention also relates to the conjugates themselves and methods of using such conjugates in treating patients. Such treatments may include, but are not limited to, modalities where delivery of nucleic acids, nucleosides, proteins, peptides, amino acid residues, lipids, carbohydrates, synthetic organic compounds, metals, vitamins, small molecules, dyes, isotopes, antibodies, toxins ligands or any other compound that may need transport into a cell is required. In one embodiment, conjugates may be administered to patients having bacterial or viral infections, including administering conjugates comprising guanidinoglycosides in amounts sufficient to inhibit or prevent such infections. In a related aspect, conjugates containing RTIs for the treatment of HIV are also envisaged.

BRIEF DESCRIPTION OF THE DRAWINGS

[00012] FIG. 1 illustrates a representative structure of a three component conjugate showing: 1) guanidine-neomycin B as a cellular carrier and RRE binder, 2) AZT monophosphate and 3) a releaseable linker.

[00013] FIG. 2 illustrates the structures for the aminoglycosides and guanidionoglycosides conjugates used to evaluate cellular uptake.

[00014] FIG. 3 presents FACS histograms showing the fluorescence intensity versus cell count for 10,000 individual 10T1/2 cells following a 1hr incubation with 0.5μM of: A) tobra-BODIPY (Red) and guanidine-tobra-BODIPY (White). B) neo-BODIPY (Red) and guanidine-neo-BODIPY (White). C) BODIPY-Cys$(Arg)_9$ (Red), or guanidine-neo-BODIPY (White). D) uptake of BODIPY-Cys$(Arg)_9$ inhibited by guanidine-neomycin B (6) at 0 μM (Red), 10 μM (Black), 200 μM (Green).

[00015] FIG. 4 illustrates cellular uptake of the fluorescein-labeled aminoglycosides and guanidinoglycosides into 10T1/2 cells that are adhered to culture plates. For each sample, both fluorescence emission (B, D, F, H, J, M) and white light differential interference contrast (A, C, E, G, I, L) are shown. Each sample is treated with 1 μM of each compound for 1 hr, washed two times with buffer and imaged (as described in Example 1). Shown are: amino tobramycinfluorescein (A and B), guanidine-tobra-fluorescein (C and D), amino-neo-BODIPY (E and F), guanidine-neo-fluorescein (G and H), fluorescein-CR_9 (I and J) and the control dye "βMe-fluorescein" (L and M).

[00016] FIG. 5 illustrates structures of fluorescein-containing compounds.

[00017] FIG. 6 shows an example of distribution type 1 (diffuse cytoplasmic, nuclear and nucleolar localization). 0.5 μm cross-sectional images of a single HeLa cell in solution are shown.

[00018] FIG. 7 shows an example of distribution type 2 (mainly nuclear and nucleolar localization). 0.5 μm cross-sectional images of a single HeLa cell in solution are shown.

[00019] FIG. 8 shows microscopy experiments for fluorescein- and BODIPY-labeled guanidinoglycosides. A) and B) show cross-sectional images of two individual HeLa cells in solution following a 30 minute treatment with 5 μM of guanidine-neo-BODIPY and cleavage with trypsin. C) Two neighboring 10T1/2 cells growing on a culture plate following a 1 hr exposure to 1 μM of 4.

DETAILED DESCRIPTION OF THE INVENTION

[00020] The present invention provides guanidinoglycoside-containing conjugates which exhibit enhanced cellular uptake at target cells. Such conjugates are useful in the delivery of therapeutic compounds for a number of diseases and disorders including, but not limited to, viral infections (e.g., retroviral infections associated with HIV, HBV, and the like), bacterial infections, and disorders associated with, for example, inappropriate mitogenic signaling, non-insulin-dependent diabetes, and inhibition of enzymes including thrombin, glycosidases, and nitric oxide synthases.

[00021] In one embodiment, the efficacy of cellular uptake for molecules conjugated to the guanidinoglycosides of the present invention is enhanced for anti HIV of nucleoside based

Reverse Transcriptase (RT) inhibitors. In a related aspect, efficacy for such RT inhibitors can be enhanced by covalently conjugating their monophosphates to guanidinoglycosides. In another embodiment, where RT inhibitors are actively transported into the cell and then released in a semi-active (and potentially fully activated) form, the necessary monophosphorylation step is circumvented. In one embodiment, essential regulatory events involving viral specific protein RNA interactions (e.g., Rev RRE) are inhibited. Thus, in a related aspect, two distinct stages in the life cycle of the virus are targeted with one anti-HIV agent. An example of this strategy is illustrated in Figure 1.

[00022] In one embodiment, the affinity of guanidinoglycosides to viral RNA sequences and their unique cellular uptake features have resulted in the formulation of a strategy where hybrid molecules containing a nucleotide analog conjugated to a guanidinoglycoside are proposed as "double warhead" anti HIV agents.

[00023] In one related aspect, the invention can be used to enhance the therapeutic factor of clinically proven nucleoside reverse transcriptase inhibitors (NRTIs) by reducing the number of metabolic activation steps needed, including but not limited to, increasing negatively charged nucleotide residence in the cell. In another aspect, NRTIs that have failed to be metabolically activated may be given new clinical applications as novel formulations. Such new and effective anti-HIV agents may be produced at low cost and low market price.

[00024] The term "alkyl" used herein refers to a monovalent straight or branched chain radical of from one to ten carbon atoms, including, but not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-hexyl, and the like. Alkyl also represents cyclic radicals, including, but not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like.

[00025] The term "cationionized" used herein refers to the process of modifying a compound with a molecule such that the surface of the compound is positively charged.

[00026] The term "perfluoroalkyl" as used herein refers to a monovalent straight chain radical of from one to four carbon atoms, in which all hydrogen atoms are substituted by fluorine. A typical perfluorinated alkyl group is the trifluoromethyl group.

[00027] The term "aryl" when used alone refers to an aromatic radical whether or not fused. Preferred aryl groups include phenyl, naphthyl, biphenyl and the like. Aryl also refers to

heteroaromatic groups including, but not limited to, furanyl, pyrrolyl, thienyl, pyrazolyl, thiazolyl, oxazolyl, pyridyl, pyrimidinyl, indolyl, and the like.

[00028] The term "substituted aryl" denotes an aryl group substituted with one, two or three substituents chosen from halogen, cyano, nitro, C_1-C_{10} alkyl, C_1-C_{10}-alkoxy, trifluoromethyl, alkoxycarbonyl, and the like. Examples of such groups are 4-chlorophenyl, 2-methylphenyl, and 3-ethoxyphenyl.

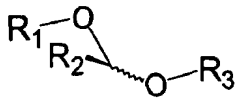
[00029] The term "arylalkyl" means one, two or three aryl groups having the designated number of carbons, appended to an alkyl chain having the number of carbons designated. A typical arylalkyl group is the benzyl group.

[00030] The term "alkenyl" refers to a straight or branched chain group of from two to ten carbon atoms containing a carbon-carbon double bond, including, but not limited to allyl, vinyl, and the like.

[00031] Also, solid supported materials, such as cation exchange resins, including materials such as sulfonated polystyrene resin may be useful.

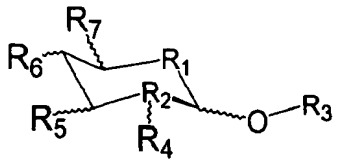
[00032] The guanidinylated cyclic acetal reagents discussed below are useful in the synthesis of conjugates for the transport/uptake of compounds into eucaryotic cells. Guanidinylating reagents and general methods for producing guanidinoglycosides are described in U.S. Pat. No. 6,525,182, the disclosure of which is herein incorporated by reference in its entirety.

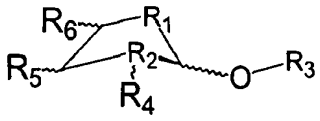
[00033] The general structure of an acetal is shown below.



[00034] In a preferred embodiment, the structure is a "cyclic acetal" where the R_1, R_2, and/or R_3 groups comprise two (or more) 5- or 6-membered rings that are linked together by at least one acetal-type functional group where R_1-R_2, and R_3 are the carbon atoms of two separate ring systems.

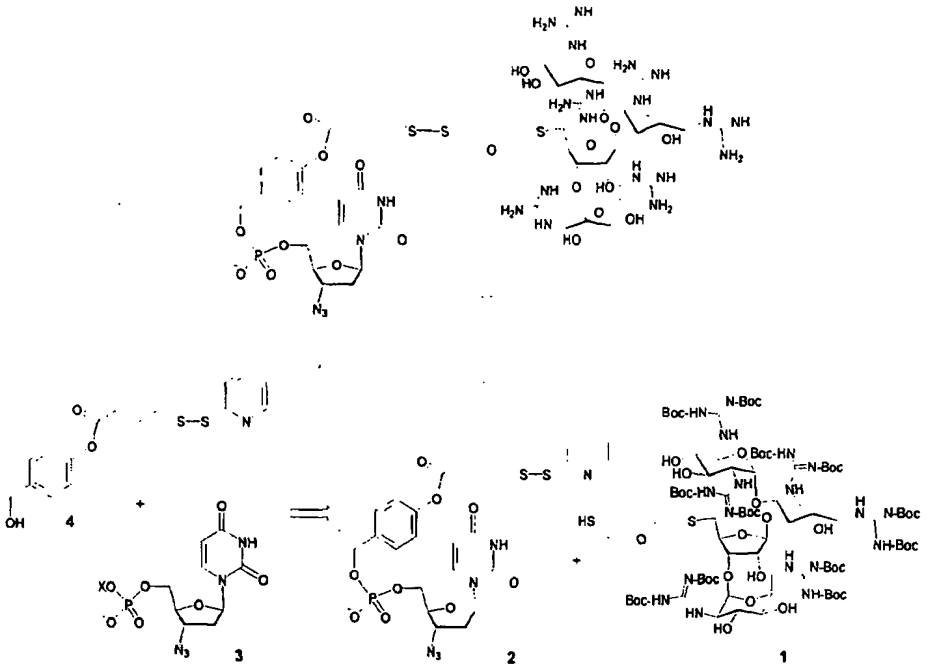
[00035] In the generic structures shown below, two or more rings are linked by at least one cyclic acetal, where R_1 and R_2 must be either carbon or oxygen atoms that compose a natural or synthetic glycoside (cyclic acetal), including, but not limited to, aminoglycosides, cardiac glycosides, dissacharides, or other polysacchrides.





[00036] In one embodiment, R_3 is a 5 or 6-membered ring (or series of rings linked by acetal linkages) that is either an additional glycosidic unit(s), or alternatively, a substituted hexane or pentane ring (where both the R_1 and R_2 groups are carbon atoms). One or more of the carbon atoms that compose these 5- or 6-membered rings must be directly substituted with one or more basic groups, where R_4-R_7 is an amine, guanidine, methylene, or alternatively, an acetal linkage to another ring system(s) that contains one or more of these functional groups.

[00037] In one embodiment, such substituted aminoglycosides are conjugated to therapeutic compounds. A representative retrosynthetic scheme that takes advantage of reactivity patterns is illustrated below.



[00038] A fully-Boc protected guanidino-neomycin B (1) bearing a long thiol-containing linker can be conjugated via an effective thiol-exchange reaction with the extended AZT-linker conjugate (2). This "extended" AZT can be obtained by condensing the activated AZT-monophosphate (3) with the linker (4), which in turn can be obtained from commercially available building blocks via standard chemistry (e.g., SIGMA Chemical Co., St. Louis, MO). Synthesis of the various modified aminoglycosides, guanidinoglycosides and their conjugates is known in the art (see, e.g., U.S. Pat. No. 6,525,182; Wang et al., J Am Chem Soc (1997) 119:8734-8735; Wang et al., Bioorg Med Chem Lett (1997) 7:1951-1956; Kirk et al., J Am Chem Soc (2000) 122:980-981; Wang et al., Bioorg Med Chem Lett (1998) 8:3665-3670; Luedtke et al., J Am Chem Soc (2000) 122:12035-12036; and Baker et al., J Org Chem (2000) 65:9054-9058). Several alternative building blocks are discussed below.

[00039] In one embodiment, the conjugate design facilitates a modular synthesis where the various components can be separately synthesized and then coupled together in advanced stages. In a related aspect, this allows mixing-and-matching of various carriers/RRE binders with a variety of linkers and NRTI monophosphates.

[00040] The modular design of the proposed conjugates translates into significant flexibility in the synthetic approach. Numerous reagents and reaction conditions are available for each coupling step. The formation of the phosphodiester linkage between AZT monophosphate (3)

and the linker (4), for example, can be facilitated by, but is not limited to, carbonyldiimidazole or via DMAP-catalyzed carbodiimide condensation.

[00041] In one embodiment, a conjugate will possess three key functional components (see, e.g., Figure 1).

[00042] For example, for nucleoside-based RT inhibitor, as HIV reverse transcriptase (RT) is a low-fidelity DNA polymerase, it can be inhibited by nucleoside analogs that mimic deoxyribonucleoside triphosphates (dNTP), its natural substrates. In a related aspect, well-established inhibitors, such as 3′-azido-2′,3′-dideoxythymidine (AZT) in their semi-activated form are envisaged as conjugated compounds.

[00043] In a further related aspect, to effectively release the semi-activated NRTI from its carrier/RRE binder, a hydrolysable linker may be employed. For example, an esterase-induced hydrolysis followed by a facile 1,6-elimination reaction that releases the NRTI-monophosphate in its intact form can be used (see e.g., Figure 1).

[00044] In one embodiment, an RRE binder/membrane translocation vehicle is contemplated. The Rev-Response-Element (RRE) serves as the Rev-binding site responsible for the active export of unspliced and singly spliced HIV genomic RNA from the nucleus. Small organic molecules that target such unique viral RNA sites can prevent the formation of a key regulatory RNA-protein complex and interfere with viral replication. In a related aspect, the use of guanidino-neomycin B and guanidino-tobramycin is contemplated. These two derivatives have substantial affinity to the RRE (see, e.g., U.S. Pat. No. 6,525,182) and very effective cellular uptake profiles (see below).

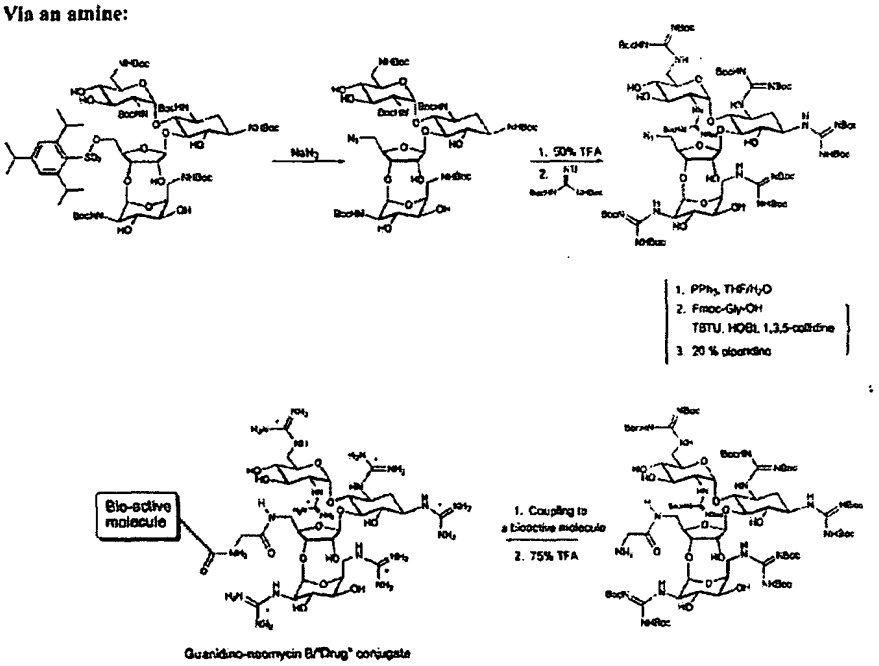
[00045] In a related aspect, any NRTI-monophosphate can potentially be employed. Similarly, various linkers with different degradation mechanisms will be readily appreciated by one of skill in the art. Additionally, numerous guanidinoglycosides can be utilized. For example, natural or synthetic guandino-sugars, or guanidinylated aminoglycoside derivatives can also be used. In one embodiment, the guanidinylated forms of dimeric aminoglycosides or the guanidylated products of aminoglycoside decomposition fragments and/or simple oligomers of these units are envisaged. In addition, modification of other (common) forms of saccharides including di-, tri-, and tetra-saccharides may also be suitable scaffolds for the presentation of guanidine groups. In one embodiment, for guanidinoglycosides, such modified forms may include, but are not limited to, guanidino-amikacin, guanidino-gentamicin, guanidino-kanamycin,

guanidino-neomycin, guanidino-netilmicin, guanidino-O-2,6-Diamino-2,6-dideoxy-beta-L-idopyranosyl-(1 to 3)-O-beta-D-ribofuranosyl-(1 to 5)-O-[2-amino-2-deoxy-alpha-D-glucopyranosyl-(1 to 4)]-2- deoxystreptamine, guanidino-streptomycin and guanidino-tobramycin.

[00046] In another embodiment, for cardiac glycosides, such modified forms may include but are not limited to, guanidino-ouabain, guanidino-deslanoside, guanidino-digoxin, guanidino-digitoxin, guanidino-lantoside, guanidino-gitoxigenin, guanidino-bufalin and guanidino-strophanthin.

[00047] For amines, a typical reaction scheme is as follows:

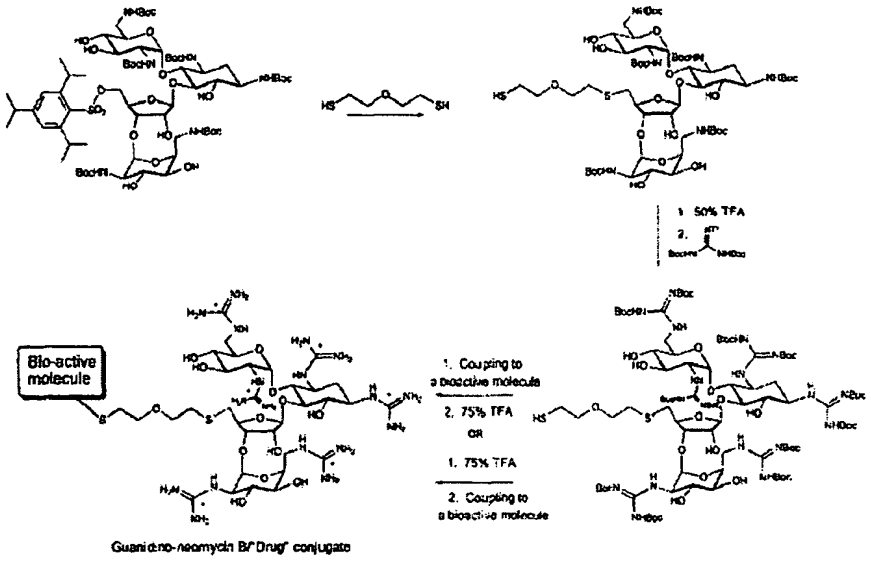
Via an amine:



[00048] In one embodiment, a general scheme for the coupling of a bioactive molecule to a guanidinylated glycoside is through an amino acid linker. In a related aspect, a 5"-TIPS activated Boc-protected neomycin B derivative is reacted with sodium azide. The Boc groups are then removed and the free amines are reacted with a Boc-protected guanidinylating reagent (see, e.g., U.S. Pat. No. 6,525,182). Thriphenol phosphine is then used to reduce the azido group into an amine. To this amine any bioactive molecule can be conjugated, with or without the use

of a linker (e.g., nucleic acids, nucleosides, proteins, peptides, amino acid residues, lipids, carbohydrates, synthetic organic compounds, metals, vitamins, small molecules, dyes, isotopes, antibodies, toxins ligands or any other compound that may need transport into a cell). In one embodiment, the amino acid glycine is used as a linker between the bioactive molecule and the guanidinylated glycoside. One of skill in the art would recognize that other amino acids may be substituted.

[00049] For thiols, a typical reaction scheme is as follows:



Guanidino-neomycin Bi*Drug* conjugate

[00050] In one embodiment, a general scheme for the coupling of a bioactive compound to a guanidinylated glycoside is through a thiol linker. In one aspect, a 5"-TIPS activated, Boc-protected neomycin derivative is reacted with a dithiol. In one embodiment, the dithiol is B-mercaptoethylether, but one of skill in the art would recognize that other such dithiols may be substituted. The Boc groups are then removed and the free amines are reacted with a Boc-protected guanidinylating reagent (see, e.g., U.S. Pat. No. 6,525,182). The coupling of the bioactive molecule through the free thiol can be done either before or after the removal of the protecting groups (in the scheme above, Boc) from the guanidine groups. This allows for the

coupling reaction to be conducted under aqueous or non-aqueous conditions (e.g., depending on the solubility and reactivity of the bioactive molecule).

[00051] In one embodiment, reversed phase HPLC is used in purifying the final product (for example, the presence of the useful thymidine chromophore for UV-detection can be exploited). In a related aspect, to facilitate the purification of intermediates, the guanidinoglycoside core is maintained in its Boc-protected form. Such building blocks are easily purified by normal phase chromatography.

[00052] As stated above, each functional component can be replaced by an alternative building block. In a related aspect, the most important parameters for advanced stages are: a) the ability to tune the cellular uptake by using different guanidinoglycosides, b) the ability to tune the linker cleavage rate by substituting the benzene core in 4,9 or use other linkers with different release mechanisms, and c) incorporate alternative nucleoside analogs.

[00053] In one embodiment, the present invention provides a method for treating a subject having a bacterial or viral infection or treating a subject susceptible to infection with a bacteria or virus. The method includes administering a guanidinoglycoside-conjugate of the invention, an analogue, derivative, or salt thereof, prior to, simultaneously with, or subsequent to infection by a bacteria or viral organism.

[00054] In another embodiment, the invention provides a method of inhibiting or modulating the progression of viral infections (e.g., retroviral infections associated with HIV, HBV, and the like), bacterial infections, and disorders associated with, for example, inappropriate mitogenic signaling, non-insulin-dependent diabetes, and inhibition of disorders associated with thrombin, glycosidases, and nitric oxide synthases.

[00055] Thus, the guanidinoglycosides-containing conjugates of the present invention, as well as analogues, derivatives, or salts thereof are useful in the treatment of various maladies in general, either separately or in combination with other therapeutically active compounds. These compounds may be administered orally, topically or parenterally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes subcutaneous injections, aerosol, intravenous, intramuscular, intrathecal, intracranial, intrastemal injection or infusion techniques.

[00056] The present invention also has the objective of providing suitable topical, oral, and parenteral pharmaceutical formulations for use in the treatment of various illnesses. The compounds of the present invention may be administered orally as tablets, aqueous or oily suspensions, lozenges, troches, powders, granules, emulsions, capsules, syrups or elixirs. The composition for oral use may contain one or more agents selected from the group of sweetening agents, flavouring agents, colouring agents and preserving agents in order to produce pharmaceutically elegant and palatable preparations. The tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, (1) inert diluents, such as calcium carbonate, lactose, calcium phosphate or sodium phosphate; (2) granulating and disintegrating agents, such as corn starch or alginic acid; (3) binding agents, such as starch, gelatin or acacia; and (4) lubricating agents, such as magnesium stearate, stearic acid or talc. These tablets may be uncoated or coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. Coating may also be performed using techniques described in the U.S. Pat. Nos. 4,256,108; 4,160,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

[00057] The guanidinoglycoside-conjugates of the invention (including analogues, derivatives, or salts thereof) can be administered, for in vivo application, parenterally by injection or by gradual perfusion over time independently or together. Administration may be intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally. For in vitro studies the agents may be added or dissolved in an appropriate biologically acceptable buffer and added to a cell or tissue.

[00058] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for

example, antimicrobials, anti-oxidants, chelating agents, growth factors and inert gases and the like.

[00059] The labels in the present invention can be primary labels (where the label comprises an element which is detected directly) or secondary labels (where the detected label binds to a primary label, e.g., as is common in immunological labeling). An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden (1997) Introduction to Immunocytochemistry second edition, Springer Verlag, N.Y. and in Haugland (1996) Handbook of Fluorescent Probes and Research Chemicals a combined handbook and catalogue Published by Molecular Probes, Inc., Eugene, Oreg. Primary and secondary labels can include undetected elements as well as detected elements. Useful primary and secondary labels in the present invention can include spectral labels such as fluorescent dyes (e.g., fluorescein and derivatives such as fluorescein isothiocyanate (FITC) and Oregon Green™, rhodamine and derivatives (e.g., Texas red, tetrarhodintine isothiocynate (TRITC), and the like), dixogenin, biotin, phycoerythrin, AMCA, CyDyes™, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, ^{33}P, and the like), enzymes (e.g., horse-radish peroxidase, alkaline phosphatase, and the like) spectral calorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, and the like) beads. The label may be coupled directly or indirectly to Rev according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions. In general, a detector is adapted to the particular label which is used. Typical detectors include X-ray machines, CAT scanners, NMR, spectrophotometers, phototubes and photodiodes, microscopes, scintillation counters, cameras, film and the like, as well as combinations thereof. Examples of suitable detectors are widely available from a variety of commercial sources known to persons of skill.

[00060] Preferred labels include those which utilize 1) chemiluminescence (using horseradish peroxidase and/or alkaline phosphatase with substrates that produce photons as breakdown products) with kits being available, e.g., from Molecular Probes, Amersham, Boehringer-Mannhiem and Life Technologies/Gibco BRL; 2) color production (using both horseradish peroxidase and/or alkaline phosphatase with substrates that produce a colored precipitate; kits available from Life Technologies/Gibco BRL, and Boehringer-Mannheim); 3) hemifluorescence using, e.g., alkaline phosphatase and the substrate AttoPhos (Amersham) or other substrates that produce fluorescent products, 4) Fluorescence (e.g., using Cy-5 (Amersham), fluorescein, and

other fluorescent tags); and 5) radioactivity. Other methods for labeling and detection will be readily apparent to one skilled in the art.

[00061] Fluorescent labels are highly preferred labels, having the advantage of requiring fewer precautions in handling, and being amendable to high-throughput visualization techniques (optical analysis including digitization of the image for analysis in an integrated system comprising a computer). Preferred labels are typically characterized by one or more of the following: high sensitivity, high stability, low background, low environmental sensitivity and high specificity in labeling. Fluorescent moieties, which are incorporated into the labels of the invention, are generally known, including BODIPY, Texas red, dixogenin, biotin, 1- and 2-aminonaphthalene, p,p'-diaminostilbenes, pyrenes, quaternary phenanthridine salts, 9-aminoacridines, p,p'-diaminobenzophenone imines, anthracenes, oxacarbocyanine, merocyanine, 3-aminoequilenin, perylene, bis-benzoxazole, bis-p-oxazolyl benzene, 1,2-benzophenazin, retinol, bis-3-aminopyridinium salts, hellebrigenin, tetracycline, sterophenol, benzimidazolylphenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, calicylate, strophanthidin, porphyrins, triarylmethanes, flavin and many others. Many fluorescent tags are commercially available from the SIGMA chemical company (St. Louis, Mo.), Molecular Probes, R&D systems (Minneapolis, Minn.), Pharmacia LKB Biotechnology (Piscataway, N.J.), CLONTECH Laboratories, Inc. (Palo Alto, Calif.), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, Wis.), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersberg, Md.), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), and Applied Biosystems (Foster City, Calif.), as well as many other commercial sources known to one of skill.

[00062] The following examples are intended to illustrate but not limit the invention.

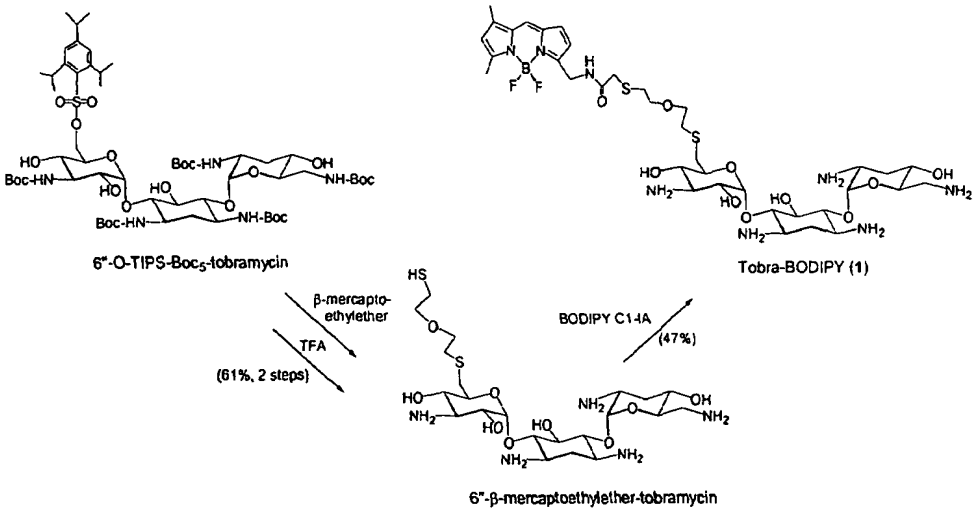
EXAMPLES

EXAMPLE 1 CELLULAR UPTAKE

[00063] To examine how the cellular uptake of conjugated compounds is affected by guanidinylation, a series of BODIPY-tagged aminoglycosides and guanidinoglycosides were synthesized based upon tobramycin and neomycin B (see, e.g., FIG. 2). BODIPY is an excellent fluorescent probe for cellular uptake studies since its fluorescence is relatively insensitive to

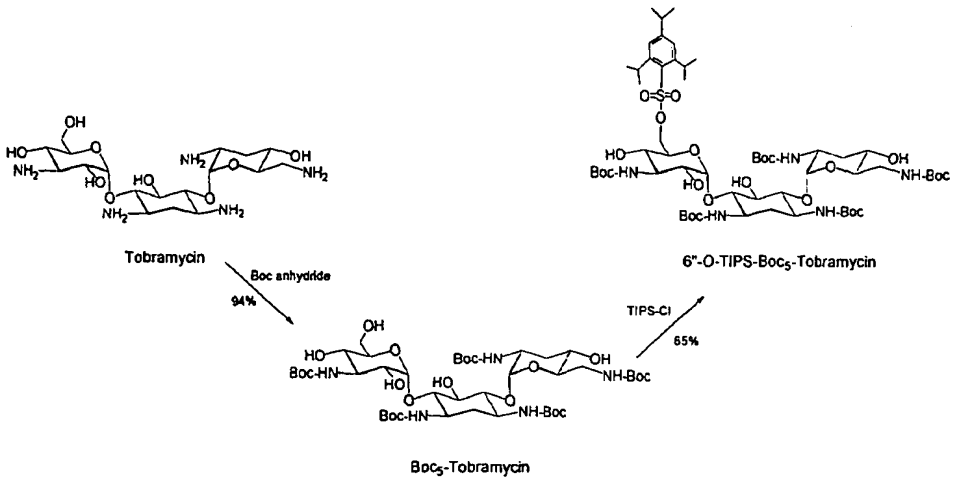
changes in the local environment. By using fluorescein as a reference (φ=0.93 at pH 9.0), the emission quantum efficiently (φ) of all five BODIPY conjugates 1 – 5 is equal to 1.0 at pH 7.5.

[00064] A scheme for synthesizing tobra-BODIPY is shown below:



[00065] Synthesis and Characterization of Tobra-BODIPY.

[00066] 6"-O-TIPS-Boc$_5$-Tobramycin. A synthesis scheme is provided below:



[00067] Boc_5-Tobramycin: A solution of tobramycin (0.5 g, 1.070 mmol) in 14 mL aqueous DMSO (DMSO : water = 6 : 1) was treated with di-tert-butyldicarbonate (1.4 g, 6.420 mmol, 6.0 equiv). The solution was heated at 60 °C for 4 hours, then cooled to 23 °C. A solution of 30% aqueous ammonia (5 mL) was added dropwise to the mixture. The precipitated solid was filtered, washed with water (3 X 200 mL), and dried in vacuo (970 mg, 94%): Rf 0.31, 7.5% methanol-dichloromethane; ^{1}H NMR (500 MHz, methanol-$_{d4}$) δ 5.10 (br, 1H), δ 5.07 (br, 1H), δ 3.93 (m, 1H), δ 3.78 (m, 1H), δ 3.70 (m, 2H), δ 3.60 (m, 3H), δ 3.30-3.50 (m, 9H), δ 2.11 (m, 1H), δ 1.99 (m, 1H), δ 1.64 (q 1H, J = 12.5 Hz), δ 1.42-1.48 (m, 46H); HRMS (FAB) m/z calcd for $C_{43}H_{77}NaN_5O_{19}$ [M+Na]+ 990.5110, found 990.5102.

[00068] 6"-O-TIPS-Boc_5-Tobramycin: A solution of Boc_5-tobramycin (0.3 g, 0.310 mmol) in pyridine (5 mL) was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (0.66g, 2.180 mmol, 7.0 equiv.). The reaction mixture was stirred at 23 °C for 12 hours. It was neutralized by adding hydrochloric acid (1.0 N), and partitioned between water (300 mL) and ethyl acetate (600 mL). The aqueous layer was separated and extracted with ethyl acetate (2 X 250 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (2.3% methanol-dichloromethane) afforded the desired product as a white solid (240 mg, 65%): Rf 0.33, 7.5% methanol-dichloromethane; ^{1}H NMR (500 MHz, methanol-$_{d4}$) δ 7.28 (s, 2H), δ 5.09 (br, 2H), δ 4.40 (m, 1H), δ 4.27 (m, 1H), δ 4.14 (m, 3H), δ 3.72 (t, 1H, J = 10.4 Hz), δ 3.40-3.60 (m, 12H), δ 2.94 (m, 1H), δ 2.04 (m, 2H), δ 1.64 (q, 1H, J = 12.0 Hz),

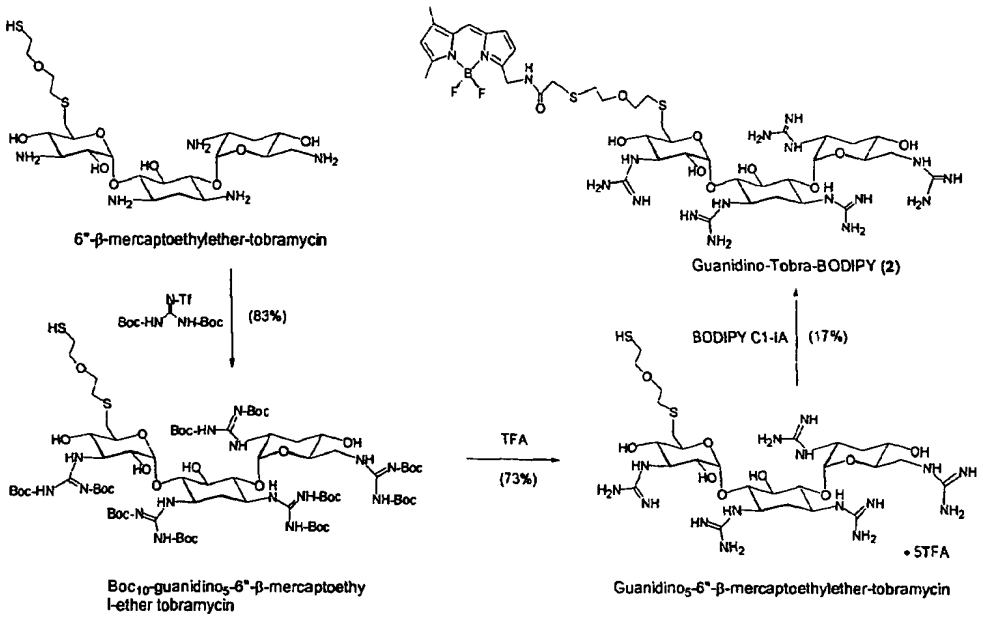
δ 1.42-1.48 (m, 46H), δ 1.26 (m, 18H), HRMS (FAB) m/z calcd for $C_{58}H_{99}NaN_5O_{21}S$ [M+Na]+ 1256.6451, found 1256.6487.

[00069] 6"-β-Mercaptoethylether-Tobramycin · TFA_5: 6"-O-TIPS-Boc_5-tobramycin (40 mg, 32 μmoles) Cs_2CO_3 (21 mg, 64 μmoles), dry DMF (3 mL), and 2-mercaptoethylether (34 μl, 274 μmoles, 8.6 equiv) were stirred under argon for 2h at 30°C, diluted into ethyl acetate (100 mL), washed with water (4 x 50 mL) and brine (50 mL), then dried over sodium sulfate. The organic layer was then concentrated under to an oil reduced pressure, and kept under a high vacuum for 40 min. The crude product was dissolved in CH_2Cl_2 (2 mL), 1,2-ethanedithiol (15 μL), triisopropysilane (15 μL), and trifluoroacetic acid (3 mL), and stirred at RT for 15 min. The reaction was then diluted into toluene (50 mL) and concentrated to a solid at 50 °C under reduced pressure (2x). The white solid was then dissolved into water and (100 mL) and washed with $CHCl_3$ (4 x 50 mL). The aqueous layer was concentrated under reduced pressure and lyophilized (2x) from 0.1% TFA (3 mL in water) to yield 25 mg of a white powder (61% yield, two steps). 1H-NMR (400 MHz, D_2O) δ 5.59 (d, J = 3.6 Hz, 1H), δ 4.85 (d, J = 4.0 Hz, 1H), δ 3.3-3.8 (m, 17H), δ 3.05 (d,d J_1 = 13.6 Hz, J_2 = 7.2 Hz, 1H), δ 2.94 (d,d J_1 = 11.6 Hz, J_2 = 2.4 Hz, 1H), δ 2.65-2.71 (m, 3H), δ 2.57 (t, J = 6.4 Hz, 2H), δ 2.40 (d,t J_1 = 12.8 Hz, J_2 = 4.0 Hz, 1H), δ 2.14 (d, t J_1 = 12.4 Hz, J_2 = 4.4 Hz, 1H), δ 1.87 (q, J = 11.2 Hz, 1H), δ 1.78 (q, J = 12.8 Hz, 1H). ESI MS calculated for $C_{22}H_{45}N_5O_9S_2$: 587.3, found 588.2 $[M+H]^+$.

[00070] Tobra-BODIPY · HCl (1): 6"-β-mercaptoethylether-tobramycin · TFA_5 (5 mg, 4.3 μmoles) was dissolved in a degassed aqueous buffer (1 mL of 150 mM NaCl, 10 mM sodium phosphate pH 7.5, Ar sparged). Separately, BODIPY C1-IA (2.5 mg, 6 μmoles, 1.4 equiv., Molecular Probes) was dissolved in DMSO (0.75 mL), and added, dropwise, to the tobramycin solution. The reaction was kept in the dark for 2h at RT then diluted into water (8 mL) and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack). The column was activated with 10 mL acetonitrile, 10 mL of water, the crude reaction was then loaded, washed with 1M NaCl (5 mL) and pure water (5 mL), then a 0 – 30% acetonitrile/water gradient was applied, and the fractions between 5 – 15% acetonitrile/water were collected and lyophilized to yield 2.2 mg (47%) of a red powder. All BODIPY-glycoside conjugates are slightly to moderately hygroscopic, therefore the absorption at 502 nm of each compound (in methanol) is used to confirm the yield of the conjugation reaction (taking ε502 nm = 76,000 cm^{-1} M^{-1}). ^{1}H-NMR (400 MHz, D_2O) δ 7.39 (s, 1H), δ 6.91 (d, J = 4.0 Hz, 1H), δ 6.30 (d, J = 4.0 Hz, 1H), δ 6.22 (s, 1H), δ 5.55 (d, J = 3.6 Hz, 1H), δ 4.94 (d, J = 4.0 Hz, 1H), δ 4.49 (s, 2H), δ 3.25-3.90 (m,

19H), δ 3.05 (d, d J_1 = 13.6 Hz, J_2 = 7.2 Hz, 1H), δ 2.93 (d,d J_1 = 11.6 Hz, J_2 = 2.4 Hz, 1H), δ 2.60-2.67 (m, 5H), δ 2.40 (s, 3H), δ 2.29 (d, t J_1 = 12.4 Hz, J_2 = 3.6 Hz, 1H), δ 2.10-2.14 (m, 4H), δ 1.84 (q, J = 11.6 Hz, 1H), δ 1.66 (q, J = 12.8 Hz, 1H). MALDI TOF MS calculated for $C_{36}H_{59}BF_2N_8O_{10}S_2$: 876.3 found 877.4 $[M+H]^+$, found 899.3 $[M+Na]^+$ found 915.4 $[M+K]^+$.

[00071] A scheme for synthesizing guanidino-tobra-BODIPY is shown below:



[00072] Synthesis and Characterization of Guanidino-Tobra-BODIPY.

[00073] Boc_{10}-$Guanidino_5$-6"-β-Mercaptoethylether-Tobramycin: 6"-β-mercaptoethylether tobramycin · TFA_5 (70 mg, 60 μmoles), was dissolved in methanol (4mL) and treated with N,N'-di-Boc-N"-trifylguanidine (420 mg, 1.08 mmoles, 17.9 equiv.), dithiothreitol (42 mg, 272 μmoles), and triethylamine (210 μL, 1.5 mmoles, 25 eqiv.) for 26 h at RT under argon. The reaction was then diluted into 150 mL of $CHCl_3$ and washed with 0.1M citric acid (3 x5 0 mL) and brine (50 mL), then dried over sodium sulfate. The organic layer was concentrated to a solid and purified on silica gel using flash chromatography and 0 – 2% methanol in CH_2Cl_2 to afford 90 mg of an off-white solid (83% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.50 (s, 1H), δ 11.47 (s, overlapping, 2H), δ 11.45 (s, 1H), δ 11.38 (s, 1H), δ 8.86 (d, J = 3.6 Hz, 1H), δ 8.55 (d, J =9.0 Hz, 1H), δ 8.46 (t, J = 6.3 Hz, 1H), δ 8.17 (d, J = 8.7 Hz, 1H), δ 5.30-5.40 (m, 2H), δ 4.97 (d, J = 3.9 Hz, 1H), δ 4.02 (br d, d J_1 = 12 Hz, J_2 = 8.4 Hz, 1H), δ 3.78-3.94 (m, 2H), δ 3.31-3.72 (m, 10H), δ 3.18 (br d, J_1 = 11.4 Hz, 2H), δ 2.95-3.03 (m, 2H), δ 2.61-2.73 (m, 8H), δ 2.36 (s, 1H), δ

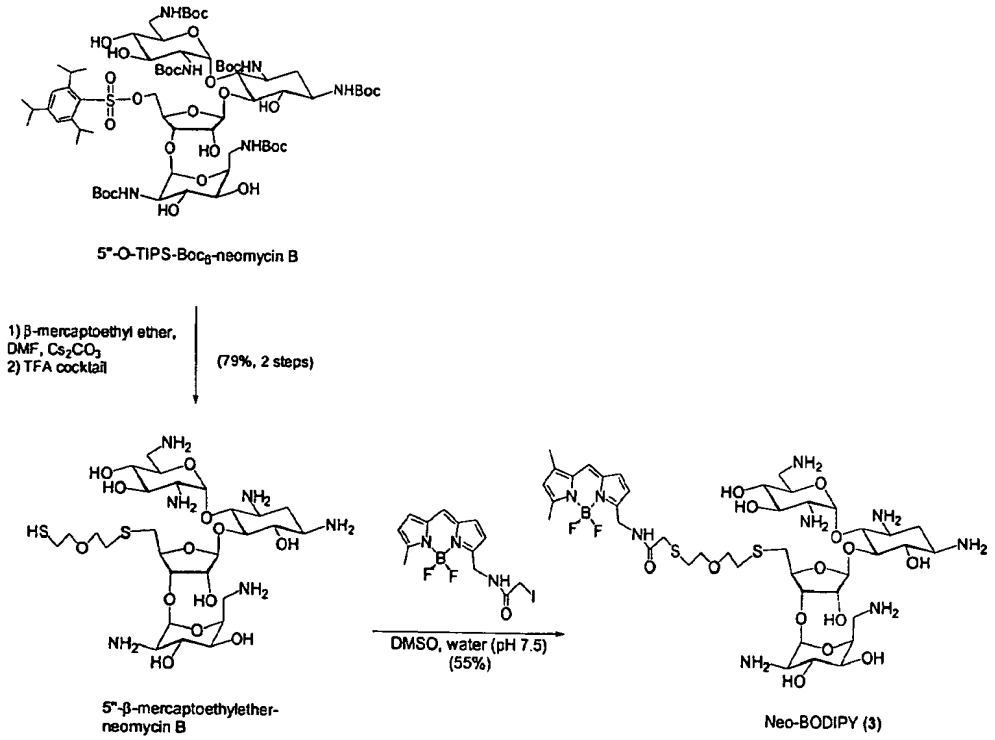
2.02 (s, 1H), δ 1.93 (s, 1H), δ 1.62-1.69 (m, 18H), δ 1.42-1.51 (m, 18H), δ 1.28 (s, 1H). ESI MS calculated for $C_{77}H_{135}N_{15}O_{29}S_2$: 1797.8, found 1798.3 $[M+H]^+$, found 899.7 $[M+2H]^{2+}$.

[00074] Guanidino$_5$-6"-β-Mercaptoethylether-Tobramycin · TFA_5: Boc_{10}-guanidino$_5$-6"-β-mercaptoethyelther-tobramycin (41 mg, 23 μmoles) was dissolved in $CHCl_3$ (1 mL) and treated with triisopropysilane (30 μL, 146 μmoles), 1,2-ethanedithiol (30 μL, 358 μmoles), and trifluoroacetic acid (1.5 mL) for 3 h at RT. The reaction was then diluted into water (100 mL) and washed with $CHCl_3$ (2 x 30 mL) and diethyl ether (2 x 30 mL). The aqueous layer was concentrated to a solid under vacuum, then dissolved in 0.1% trifluoroacetic acid in water (2 mL) and lyophilized to afford 22 mg of a white solid (73% yield). ^{1}H-NMR (400 MHz, d_6-MeOD) δ 5.65 (d, J = 3.6 Hz, 1H), δ 5.06 (d, J = 3.6 Hz, 1H), δ 4.10 (t, J = 6.4 Hz, 1H), δ 3.45-3.88 (m, 17H), δ 3.04 (d,d J_1 = 13.6 Hz, J_2 = 2.8 Hz, 1H), δ 2.62-2.78 (m, 5H), δ 2.11-2.19 (m, 2H), δ 1.68-1.78 (m, 2H). MALDI TOF MS calculated for $C_{27}H_{55}N_{15}O_9S_2$: 797.37, found 820.32 $[M+Na]^+$.

[00075] Guanidino-Tobra-BODIPY · HCl (2): Guanidino$_5$-6"-β-mercaptoethylether-tobramycin · TFA_5 (10 mg, 4.3 μmoles) was added to an aqueous degassed buffer (2 mL of 50 mM sodium phosphate pH 7.5, Ar sparged). Separately, BODIPY C1-IA (2.5 mg, 6 μmoles, 1.4 equiv., Molecular Probes) was dissolved in DMSO (0.75 mL), and added, dropwise, to the tobramycin solution. The resulting precipitation of guanidino-tobramycin was partially reversed upon addition of NaCl (150 mM final concentration). The reaction was then kept in the dark for 2h at RT and diluted into 5% acetonitrile in water (15 mL, containing 100 mM NaCl) and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack). The column was then washed with 5 mL of water and the product was eluted with 25% acetonitrile/water and lyophilized to yield 1.3 mg (17% yield) of a red powder. All BODIPY-glycoside conjugates are slightly to moderately hygroscopic, the absorption at 502 nm (in methanol) was used to calculate the yield of the conjugation reaction (taking ε502 nm = 76,000 cm^{-1} M^{-1}). The low yield of this particular reaction was attributed to the solubility problems of the guanidino-tobramycin starting material in 50 mM phosphate/25% DMSO in water (its solubility in 10 mM sodium phosphate pH 7.5, 250 mM NaCl, 25% DMSO is, however, significantly better). ^{1}H-NMR (400 MHz, D_2O) δ 7.41 (s, 1H), δ 6.92 (d, J = 3.6 Hz, 1H), δ 6.31 (d, J = 3.6 Hz, 1H), δ 6.24 (s, 1H), δ 5.40 (d, J = 3.6 Hz, 1H), δ 4.98 (s, 1H), δ 4.51 (s, 2H), δ 4.03 (t, J = 6.8 Hz, 1H), δ 3.79 (t, J = 8.4 Hz, 1H), δ 3.27-3.64 (m, 18H), δ 2.90 (d, J = 13.2 Hz, 1H), δ 2.55-2.67 (m, 5H), δ 2.41 (s, 3H), δ 2.02-2.15

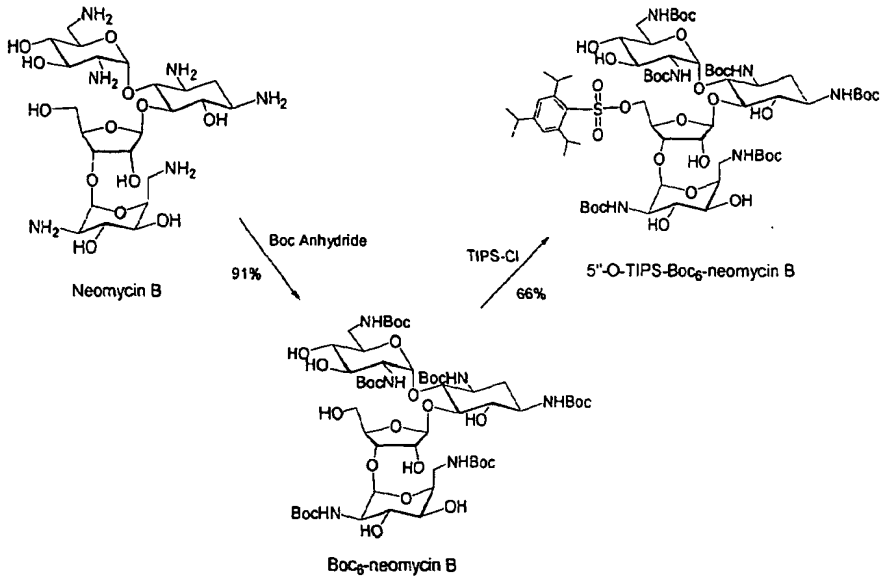
(m, 5H), δ 1.54-1.62 (m, 2H). MALDI TOF calculated for $C_{41}H_{69}BF_2N_{18}O_{10}S_2$:1086.49, found 1087.36 $[M+H]^+$, found 1109.30 $[M+Na]^+$.

[00076] A scheme for synthesizing neo-BODIPY is shown as follows:



[00077] Synthesis and Characterization of Neo-BODIPY.

[00078] 5"-O-TIPS-Boc_6-Neomycin B. A scheme for synthesizing 5"-O-TIPS-Boc_6-Neomycin B is provides below:



[00079] Boc_6-Neomycin B: Boc-neomycin B (39). A solution of Neomycin B (1.0 g, 1.626 mmol) in a mixture of DMF (20 mL), water (4 mL) and triethylamine (2 mL) was treated with di-tert-butylcarbonate (2.1 g, 9.756 mmol, 6.0 equiv.). The reaction solution was heated to 60 °C for 5 hours, then cooled to 23 °C. The volatiles were removed in vacuo. The residue was partitioned between water (300 mL) and ethyl acetate (600 mL). The aqueous layer was separated and extracted with ethyl acetate (2 X 250 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash column chromatography (4.3% methanol-dichloromethane) afforded the desired product as a white solid (1.8 g, 91%): Rf 0.36, 10% methanol-dichloromethane; ^{1}H NMR (500 MHz, methanol-$_{d4}$) δ 5.28 (br, 1H), δ 5.16 (s, 1H), δ 4.90 (s, 1H), δ 4.18 (s, 1H), δ 3.96 (s, 1H), δ 3.82-3.90 (m, 3H), δ 3.76 (s, 1H), δ 3.64-3.72 (m, 4H), δ 3.48 (m, 6H), δ 3.19-3.30 (m, 5H), δ 1.94 (m, 1H), δ 1.56 (m, 1H), δ 1.38-1.46 (m, 54H); HRMS (FAB) m/z calcd for $C_{54}H_{94}NaN_6O_{25}$ [M+Na]+ 1237.6166 found 1237.6141.

[00080] 5"-O-TIPS-Boc_6-Neomycin B: A solution of Boc_6-neomycin B (1.0 g, 0.823 mmol) in pyridine (20 mL) was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (8 g, 26.4 mmol, 32.0 equiv.). The reaction mixture was stirred at 23 °C for 12 hours. It was neutralized by adding hydrochloric acid (1.0 N) and partitioned between water (300 mL) and ethyl acetate (600 mL). The aqueous layer was separated and extracted with ethyl acetate (2 X 250 mL). The

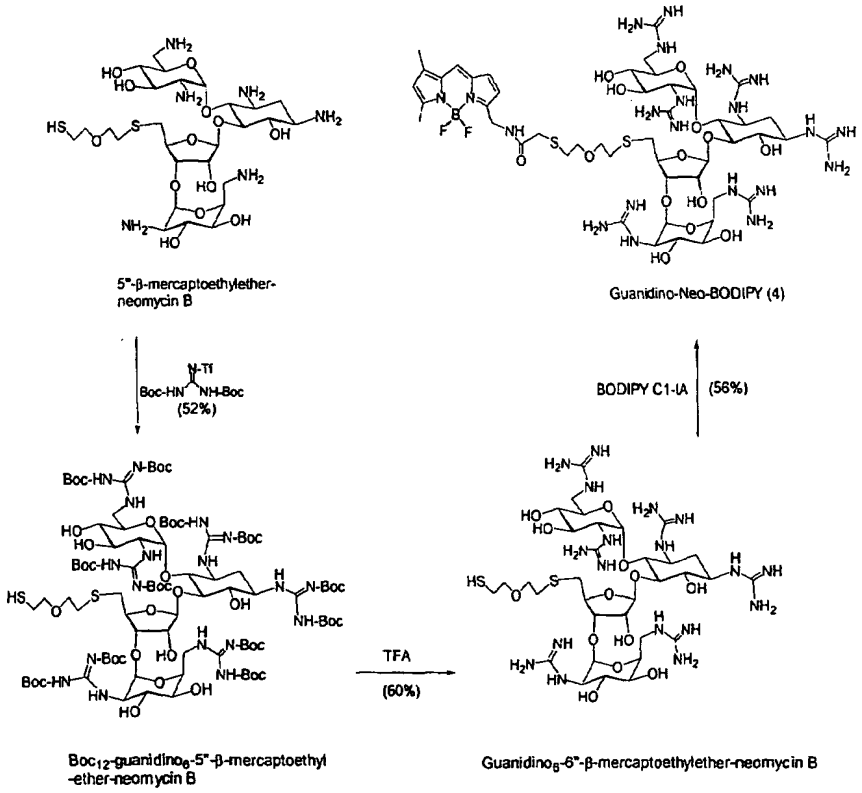
combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (3.3% methanol-dichloromethane) afforded the desired product as a white solid (0.8 g, 66%): Rf 0.40, 10% methanol-dichloromethane; ^{1}H NMR (500 MHz, methanol-$_{d4}$) δ 7.32 (2, 2H), δ 5.45 (br, 1H), δ 5.18 (br, 1H), δ 4.60 (br, 1H), δ 4.25 (m, 1H), δ 4.26 (m, 2H), δ 4.15 (m, 4H), δ 3.88 (s, 1H), δ 3.78 (m, 1H), δ 3.73 (m, 2H), δ 3.60 (m, 1H), δ 3.50 (m, 4H), δ 3.36-3.42 (m, 4H), δ 3.20 (m, 2H), δ 2.96 (m, 1H), δ 1.95 (m, 1H), δ 1.56 (m, 1H), δ 1.38-1.46 (m, 54H), δ 1.27 (m, 18H); HRMS (FAB) m/z calcd for $C_{68}H_{116}NaN_6O_{27}S$ [M+Na]+ 1503.7507, found 1503.7498.

[00081] 5"-β-Mercaptoethylether-Neomycin · TFA_6: 5"-O-TIPS-Boc_6-neomycin B (40 mg, 27 μmoles) was dissolved in DMF (1.5 mL) and treated with Cs_2CO_3 (100 mg, 307 μmoles) and 2-mercaptoethylether (125 μL, 1 mmoles, 37 equiv). The reaction was kept under argon for 7h at 30 °C, diluted into ethyl acetate (150 mL), washed with 0.1 M citric acid (50 mL), water (3 x 50 mL), brine (50 mL), and dried over sodium sulfate. The organic layer was concentrated under reduced pressure and kept under a high vacuum overnight. The crude product was dissolved in CH_2Cl_2 (4 mL) and treated with 1,2-ethanedithiol (20 μL), triisopropysilane (20 μL), and trifluoroacetic acid (5 mL) for 15 min. at RT. The reaction was diluted into toluene (50 mL) and concentrated under vacuum at 50 °C (2x) and kept under high-vacuum for 6 h. The solid was then dissolved in 0.1% TFA in water (3 mL), filtered through glass wool, and lyophilized to afford 30 mg of a white solid (79% yield, two steps). ^{1}H-NMR (400 MHz, D_2O) δ 5.88 (d, J = 4.0 Hz, 1H), δ 5.23 (d, J = 3.2 Hz, 1H), δ 5.12 (s, 1H), δ 4.21-4.24 (m, 2H), δ 4.13-4.18 (m, 2H), δ 4.04 (s, 1H), δ 3.91 (t, J = 10 Hz, 1H), δ 3.82 (t, J = 9.6 Hz, 1H), δ 3.71-3.75 (m, 2H), δ 3.64 (s, 1H), δ 3.48-3.57 (m, 4H), δ 3.17-3.41 (m, 8H), δ 3.11 (d, d J_1 = 13.6 Hz, J_2 = 9.6 Hz, 1H), δ 2.97 (d, d J_1 = 13.2 Hz, J_2 = 3.6 Hz, 1H), δ 2.65-2.74 (m, 4H), δ 2.55 (t, J = 6.0 Hz, 2H), δ 2.30 (d, t J_1 = 12.4 Hz, J_2 = 4.4 Hz, 1H), δ 1.71 (q, J = 12.4 Hz, 1H). ESI MS calculated for $C_{27}H_{54}N_6O_{13}S_2$: 734.3, found 735.3 $[M+H]^+$.

[00082] Neo-BODIPY · HCl (3): 5"-β-mercaptoethylether-neomycin B · TFA_6 (10 mg, 7 μmoles), was dissolved in an aqueous buffer (1.5 mL of 10 mM sodium phosphate, 150 mM NaCl, pH 7.5, Ar sparged). Separately, BODIPY C1-IA (1.8 mg, 4.3 μmoles, 0.61 equiv., Molecular Probes) was dissolved in DMSO (1.5 mL), and added, dropwise, to the neomycin solution and allowed to react in the dark for 2h at RT. The reaction was then diluted into water (8 mL) and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack), the column was then washed with 5% acetonitrile (5 mL, containing 100 mM NaCl in water) and then pure

water (1 mL). The product eluted between 0 – 15% acetonitrile (in water) and was lyophilized to yield 2.9 mg (55% yield) of a red powder. All BODIPY-glycoside conjugates are slightly to moderately hygroscopic, therefore the absorption at 502 nm of each compound (in methanol) is used to confirm the yield of the conjugation reaction (taking ε502 nm = 76,000 cm^{-1} M^{-1}). ^1H-NMR (400 MHz, D_2O) δ 7.41 (s, 1H), δ 6.92 (d, J = 4.0 Hz, 1H), δ 6.30 (d, J = 4.0 Hz, 1H), δ 6.24 (s, 1H), δ 5.89 (d, J = 4.0 Hz, 1H), δ 5.27 (d, J = 3.6 Hz, 1H), δ 5.15 (s, 1H), δ 4.50 (s, 2H), δ 4.26-4.32 (m, 2H), δ 4.21 (p, J = 4.0 Hz, 1H), δ 4.15 (t, J = 4.4 Hz, 1H), δ 4.07 (t, J = 3.2 Hz, 1H), δ 3.83-3.88 (m, 3H), δ 3.77 (t, J = 9.2 Hz, 1H), δ 3.67 (s, 1H), δ 3.53-3.59 (m, 4H), δ 3.45 (s, 1H), δ 3.40 (d, d J_1 = 11.2 Hz, J_2 = 4.0 Hz, 1H), δ 3.67 (s, 1H), δ 3.20-3.35 (m, 5H), δ 3.09 (d d, J_1 = 13.2 Hz, J_2 = 7.6 Hz, 1H), δ 2.98 (d d, J_1 = 13.6 Hz, J_2 = 4.4 Hz, 1H), δ 2.67-2.77 (m, 5H), δ 2.41 (s, 3H), δ 2.25 (d t, J_1 = 12.4 Hz, J_2 = 4.4 Hz, 1H), δ 2.15 (s, 3H), δ 1.65 (q, J = 12.8 Hz, 1H). MALDI TOF MS calculated for $C_{41}H_{68}BF_2N_9O_{14}S_2$: 1023.44, observed 1024.42 $[M+H]^+$, observed 1046.43 $[M+Na]^+$, observed 1062.54 $[M+K]^+$.

[00083] A scheme for synthesizing guanidine-neo-BODIPY is shown below:



[00084] Synthesis and Characterization of Guanidino-Neo-BODIPY.

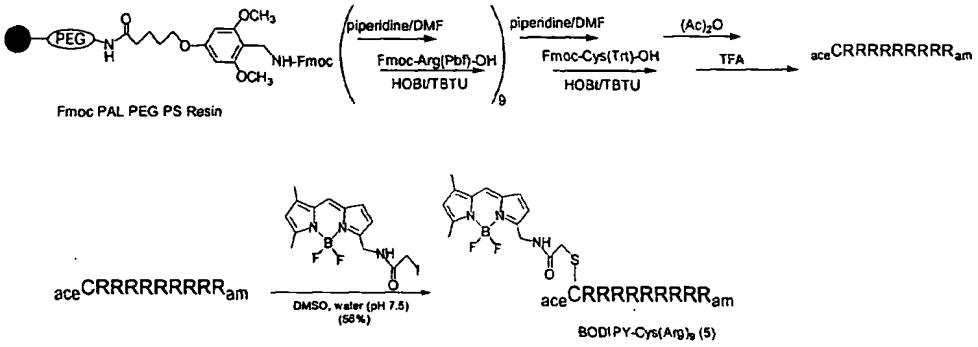
[00085] Boc_{12}-$Guanidino_6$-5"-β-Mercaptoethylether-Neomycin B: 5"-β-Mercaptoethylether-neomycin B · TFA_6 (90 mg, 63 μmoles), was dissolved in methanol (5 mL), $CHCl_3$ (3 mL), and treated with N,N'-di-Boc-N"-trifylguanidine (530 mg, 1.35 mmoles, 21 equiv.), dithiothreitol (50 mg, 324 μmoles), and triethylamine (530 μL, 3.8 mmoles, 60 eqiv.) for 96 h at RT under argon. The reaction was then diluted into $CHCl_3$ (200 mL) and washed with 0.1 M citric acid (2x100 mL), brine (50 mL) and dried over sodium sulfate. The organic layer was then concentrated to a solid under reduced pressure and purified on silica gel using flash chromatography (0-1% methanol in $CHCl_3$ to afford 71 mg of an off-white solid (52% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 11.41 (s, 2H, overlapping), δ 11.40 (s, 1H), δ 11.38 (s, 1H), δ 11.33 (s, 1H), δ 11.30 (s, 1H), δ 9.34 (d, J = 8.1 Hz, 1H), δ 8.92 (d, J = 7.2 Hz, 1H), δ 8.47 (t, J = 5.4 Hz, 1H), δ 8.41 (t, J = 5.4 Hz, 1H), δ 8.34 (d, J = 6.6 Hz, 1H), δ 8.18 (d, J = 9.0 Hz, 1H), δ 5.94 (d, J = 4.4 Hz, 1H), δ 5.62 (d, J = 4.2 Hz, 1H), δ 5.01 (d, J = 4.8 Hz, 1H), δ 4.91-4.94 (m, 2H), δ 4.38-4.95 (m, 3H), 4.05-4.22 (m, 4H), δ 3.79-3.92 (m, 3H), δ 3.66-3.74 (m, 2H), δ 3.55-3.59 (m, 4H), δ 3.25-3.42 (m, 4H), δ 2.54-2.74 (m, 5H), δ 2.44 (d t, J_1 = 12.4 Hz, J_2 = 4.4 Hz, 1H), δ 1.22-1.60 (m, 109H). ESI MS calculated for $C_{94}H_{163}N_{17}O_{37}S_2$: 2186.1, found 1094.2 $[M+2H]^{2+}$.

[00086] $Guanidino_6$-5"-β-Mercaptoethylether-Neomycin B · TFA_6. Boc_{12}-$guanidino_6$-5"-β-mercaptoethylether-neomycin B (65 mg, 30 μmoles) was dissolved in $CHCl_3$ (1.5 mL) and treated with triisopropylsilane (80 μL, 390 μmoles), 1,2-ethanedithiol (30 μL, 955 μmoles), and trifluoroacetic acid (3 mL) for 3 h at RT. The reaction was then diluted into water (200 mL) and washed with $CHCl_3$ (2 x 100 mL) and diethyl ether (2 x 50 mL). The aqueous layer was then concentrated to a solid under reduced pressure, dissolved in 0.1% trifluoroacetic acid in water (2 mL) and lyophilized to afford 30 mg of a white solid (60% yield). ^{1}H-NMR (400 MHz, D_2O) δ 5.83 (d, J = 3.2 Hz, 1H), δ 5.05 (s, 1H), δ 4.94 (s, 1H), δ 4.23-4.26 (m, 2H), δ 3.93-4.03 (m, 3H), δ 3.25-3.69 (m, 20H), δ 2.87 (d, d J_1 = 14.4 Hz, J_2 = 4.4 Hz, 1H), δ 2.55-2.67 (m, 5H), δ 2.08 (d t, J_1 = 12.0 Hz, J_2 = 4.4 Hz, 1H), δ 1.71 (q, J = 12.0 Hz, 1H). MALDI TOF MS calculated for $C_{33}H_{66}N_{18}O_{13}S_2$: 986.45, found 987.49 $[M+H]^+$.

[00087] Guanidino-Neo-BODIPY · HCl (4) $Guanidino_6$-5"-β-mercaptoethylether-neomycin B · TFA_6 (9 mg, 5.4 μmoles) was dissolved in an aqueous buffer (3.0 mL of 10 mM sodium phosphate pH 7.5, 150 mM NaCl, Ar sparged). Separately BODIPY C1-IA (1.7 mg, 4.1 μmoles, 0.76 equiv., Molecular Probes) was dissolved in DMSO (1.5 mL), and added, dropwise, to the neomycin solution and allowed to react in the dark for 2h at RT. The reaction was then diluted

into water (8 mL) and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack). The column was washed with 5% acetonitrile (5 mL containing 100 mM NaCl in water) and pure water (1 mL). The product eluted between 0 – 20% acetonitrile (in water), and was lyophilized to yield 4.5 mg (56% yield) of a red powder. All BODIPY-glycoside conjugates are slightly to moderately hygroscopic, therefore the absorption at 502 nm of each compound (in methanol) is used to confirm the yield of the conjugation reaction (taking $\varepsilon 502$ nm = 76,000 cm^{-1} M^{-1}). ^{1}H-NMR (400 MHz, D_2O) δ 7.40 (s, 1H), δ 6.92 (d, J = 4.0 Hz, 1H), δ 6.31 (d, J = 4.0 Hz, 1H), δ 6.24 (s, 1H), δ 5.84 (d, J = 3.6 Hz, 1H), δ 5.07 (s, 1H), δ 4.91 (s, 1H), δ 4.51 (s, 2H), δ 4.21-4.24 (m, 2H), δ 3.93-4.00 (m, 3H), δ 3.80 (t, J = 8.4 Hz, 1H), δ 3.66-3.71 (m, 2H), δ 3.25-3.55 (m, 15H), δ 2.80 (d, d J_1 = 14.4 Hz, J_2 = 4.4 Hz, 1H), δ 2.50-2.67 (m, 6H), δ 2.41 (s, 3H), δ 2.15 (s, 3H), δ 2.08 (d t, J_1 = 12.0 Hz, J_2 = 4.4 Hz, 1H), δ 1.57 (q, J = 12.0 Hz, 1H). MALDI TOF MS calculated for $C_{47}H_{80}BF_2N_{21}O_{14}S_2$: 1275.57, found 1276.57 $[M+H]^+$.

[00088] A scheme for synthesizing BODIPY-Cys(Arg)$_9$ is shown below:



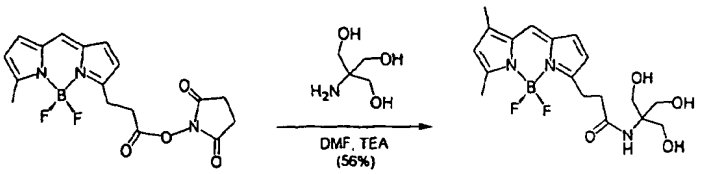
[00089] Synthesis and Characterization of BODIPY-Cys(Arg)$_9$.

[00090] ace-CRRRRRRRRR-am · TFA$_9$. Standard Fmoc solid-phase synthesis was manually employed. Fmoc PAL PEG PS resin (1 g, 0.15 mmole, PerSeptive Biosystems), was deprotected using 20% piperidine in DMF for 20 min at RT, washed with DMF (3 x 7 mL), diethyl ether (2 x 7 mL), and DMF (3 x 7 mL). The resin was then treated with TBTU (97 mg, 0.3 mmoles), Fmoc Arg (Pbf)-OH (195 mg, 0.3 mmoles), HOBt (46 mg, 0.3 mmoles), 2,4,6 collidine (0.4 mL, 3.0 mmoles), in DMF (7 mL), for at least 1 hr at RT on shaker. The resin was then washed (as

above). The deprotection and coupling processes were repeated nine times total (as above). The final coupling reaction utilized Fmoc-Cys(Trt)-OH (264 mg, 0.45 mmoles), TBTU (145 mg, 0.45 mmoles), HOBt (46 mg, 0.45 mmoles), 2,4,6 collidine (0.6 mL, 4.5 mmoles), in DMF (7 mL) and lasted for 2 h at RT on a shaker. Following deprotection and washes (as above), the terminus was acylated using HOBt (80 mg, 0.78 mmoles), diisopropylethylamine (0.9 mL), acetic anhydride (1.9 mL) in DMF (5mL) for 1 h at RT on a shaker. The resin was then washed with DMF (3 x 7 mL), diethyl ether (2 x 7 mL), and $CHCl_3$ (4 x 7 mL). The peptide was deprotected and cleaved from the resin using TFA (9 mL) in the presence of triisopropysilane (400 µL, 2 mmoles) and 1,2-ethanedithiol (0.2 mL, 6.4 mmoles) for 2.5 h at RT on a shaker. The solution was drained into 1% acetic acid/water (180 mL), and washed with $CHCl_3$ (3x80 mL) and diethyl ether (3 x 80 mL). The aqueous layer was then concentrated to a solid and lyophilized from 0.1% TFA in water. The crude peptide was purified using a 9% acetonitrile/water (0.1% TFA) isocratic mixture on a C-18 reversed phase HPLC column (3 mL/min, retention time 10-12 min) and lyophilized to afford a white solid (40 mg, 10%). MALDI TOF MS calculated for $C_{59}H_{118}N_{38}O_{115}$: 1566.96, found 1567.82 $[M+H]^+$.

[00091] BODIPY-Cys(Arg)$_9$ · HCl (5). The purified peptide ace-CRRRRRRRRR-am · TFA$_9$ (10 mg, 3.86 umoles) was dissolved in an aqueous degassed buffer (1 mL of 100 mM NaCl, 10 mM phosphate pH 7.5, Ar sparged) and treated (dropwise) with a solution of BODIPY C1-IA (1.3 mg, 3.1 µmoles, 0.81 equiv., Molecular Probes) that was pre-dissolved in DMSO (1.25 mL). The reaction was allowed to react in the dark for 1h at RT, then diluted with 8 mL of water and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack). The column was washed with 5% acetonitrile (5 mL containing 100 mM of NaCl in water) and 1 mL pure water. The product eluted between 0 – 20% acetonitrile (in water) and was lyophilized to yield 3.8 mg (56% yield) of a red powder. All BODIPY-glycoside conjugates are slightly to moderately hygroscopic, therefore the absorption at 502 nm (in methanol) of each compound was used to confirm the yield of the conjugation reaction (taking ε502 nm = 76,000 cm^{-1} M^{-1}). ^{1}H-NMR (400 MHz, D_2O) δ 7.43 (s, 1H), δ 6.91 (d, J = 3.6 Hz, 1H), δ 6.29 (d, J = 3.6 Hz, 1H), δ 6.25 (s, 1H), δ 4.53 (s, 2H), δ 4.32 (t, J = 7.2 Hz, 1H), δ 4.13-4.23 (m, 10H), δ 3.34 (s, 2H), δ 2.96-3.08 (m, 18H), δ 2.86 (d, J = 6.8 Hz, 2H), δ 2.42 (s, 3H), δ 2.17 (s, 3H), δ 1.89 (s, 3H), δ 1.50-1.71 (m, 36H). The ^{1}H NMR suggests better than 95% purity for this, and all other compounds evaluated. MALDI TOF MS calculated for $C_{73}H_{132}BF_2N_{41}O_{12}S$: 1856, found 1857 $[M+H]^+$.

[00092] A scheme for synthesizing Tris-BODIPY is shown below:



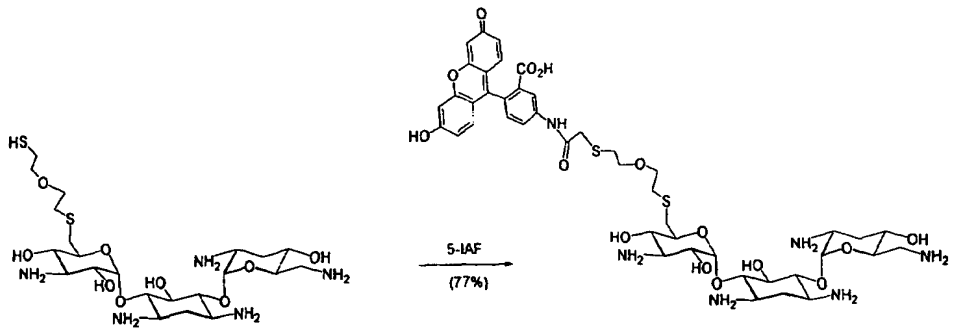
BODIPY FL SE

Tris-BODIPY

[00093] Synthesis and Characterization of Tris-BODIPY.

[00094] Tris-BODIPY. BODIPY FL SE (Molecular Probes) (~0.4 mg, ~1 μmoles) was dissolved in DMF (1 ml), and treated with Trisma base (10 mg, 126 μmoles) and triethylamine (10 μl, 72 μmoles) for 1 h at RT in the dark. The reaction was diluted into 8 ml of 50 mM phosphate pH 7.5 and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack). The column was washed with 5 ml of pure water. The desired product eluted with 30% acetonitrile (in water), and was lyophilized to yield ~0.2 mg (56% yield) of a red powder. All BODIPY-glycoside conjugates are slightly to moderately hygroscopic, therefore the absorbance at 502 nm (in methanol) of each compound is used to calculate the yield of the conjugation reaction (taking ε502 nm = 76,000 cm^{-1} M^{-1}). ^{1}H-NMR (400 MHz, MeOD) δ 7.43 (s, 1H), δ 7.00 (d, J = 4.0 Hz, 1H), δ 6.35 (d, J = 4.0 Hz, 1H), δ 6.21 (s, 1H), δ 3.71 (s, 6H), δ 3.21 (t, J = 8.0 Hz, 2H), δ 2.68 (t, J = 8.0 Hz, 2H), δ 2.50 (s, 3H), δ 2.28 (s, 3H), ESI MS calculated for $C_{18}H_{24}BF_2N_3O_4$: 395.2, found 418.3 $[M+Na]^+$.

[00095] Synthesis and Characterization of Amino-Tobra-Fluorescein:

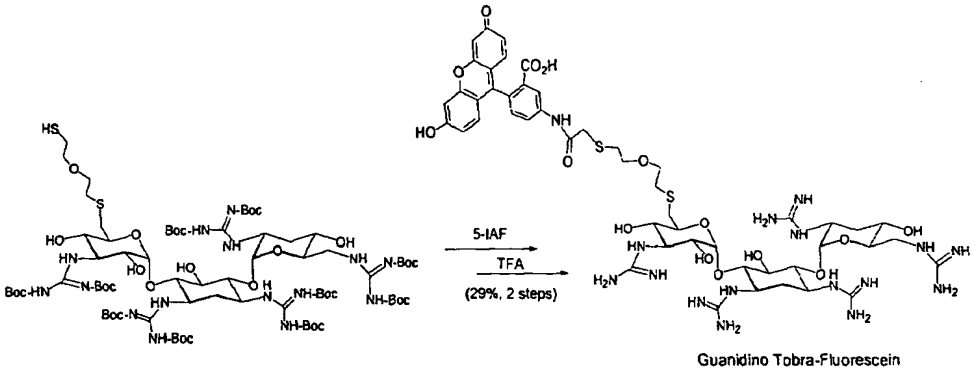


6"-β-mercaptoethylether-Tobramycin

Amino Tobra-Fluorescein

[00096] **Amino Tobra-Fluorescein · TFA_5**: 6"-β-mercaptoethyl ether tobramycin · TFA_5 (3 mg, 2.6 μmoles) was dissolved in an aqueous degassed buffer (2 mL of 400 mM NaCl, 25 mM sodium phosphate pH 7.5, Ar sparged), separately 5-iodo-acetamido-fluorescein (5-IAF) (3.0 mg, 5.8 μmoles, 2.0 equiv., Molecular Probes) was dissolved in DMSO (1 mL), and added, dropwise, to the tobramycin solution and allowed to react in the dark for 2h at RT then 0.1M HCl was added until the solution turned from orange to yellow. The reaction was then diluted into water (8 mL) and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack), the column was then washed with pure water (10 mL), and the product was eluted with 20% acetonitrile/water, lyophilized, and found to be >95% pure by HLPC. The product was purified further using a C-18 reversed phase HPLC column with an isocratic mixture of 20% acetonitrile (0.1% TFA) in water (0.1% TFA) (3 mL/min) (R_t = 8.5 min) to yield 3.3 mg (77%) of an orange solid. All fluorescein-glycoside conjugates are slightly to moderately hygroscopic, therefore the absorption at 496nm (in aqueous buffer pH 9.0) of each compound is used to confirm the yield of the conjugation reaction (taking $\varepsilon_{502\ nm}$ = 77,000 cm^{-1} M^{-1}). ^{1}H-NMR (400 MHz, D_2O) δ 8.13 (d, J = 2.0 Hz, 1H), δ 7.67 (d,d J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), δ 7.15-7.22 (m, 3H), δ 6.93 (d, J = 2.4 Hz, 2H), 6.77-6.80 (m, 2H), δ 5.53 (d, J = 2.8 Hz, 1H), δ 4.85 (d, J = 3.6 Hz, 1H), δ 3.22-3.83 (m, 21H), δ 3.05 (d,d J_1 = 13.2 Hz, J_2 = 7.2 Hz, 1H), δ 2.87 (d,d J_1 = 11.6 Hz, J_2 = 2.0 Hz, 1H), δ 2.77 (t, J = 5.8 Hz, 2H), δ 2.57-2.65 (m, 3H), δ 2.36 (d,t J_1 = 12.0 Hz, J_2 = 3.6 Hz, 1H), δ 2.09 (d,t J_1 = 12.4 Hz, J_2 = 3.6 Hz, 1H), δ 1.83 (q, J = 11.6 Hz, 1H), δ 1.74 (q, J = 12.4 Hz, 1H). MALDI TOF MS calculated for $C_{44}H_{58}N_6O_{15}S_2$: 974.34 found 975.42 $[M+H]^+$, found 997.43 $[M+Na]^+$ found 1013.41 $[M+K]^+$.

[00097] Synthesis and Characterization of GuanidinoTobra-Fluorescein:



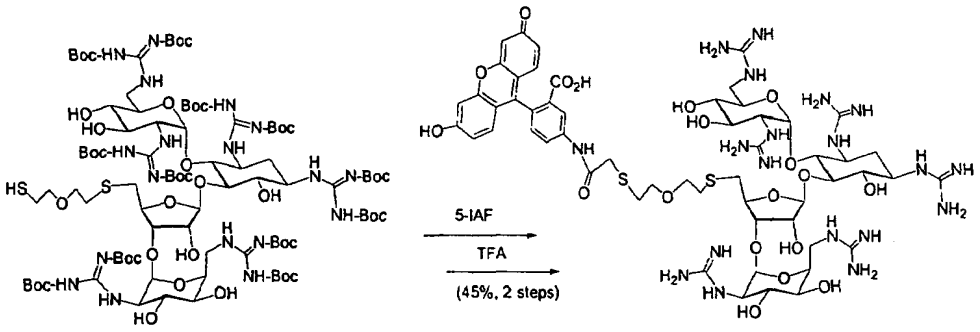
Boc_{10}Guanidino$_5$-6"-β-Mercaptoethyl -ether Tobramycin

Guanidino Tobra-Fluorescein

[00098] **Guanidino Tobra-Fluorescein · TFA_5**: Boc_{10} guanidino$_5$ 6" β-mercaptoethyl ether tobramycin (10 mg, 5.6 μmoles), DMF (3 mL), Cs_2CO_3 (30 mg), and 5-iodo-acetamido-fluorescein (5-IAF) (5 mg, 9.7 μmoles, 1.7 equiv., Molecular Probes) were stirred at RT in the dark for 2h then diluted into ethyl acetate (150 mL) and washed with 1M Na_2CO_3 (2x50 mL), 0.1M citric acid (2x50 mL), brine (50 mL), dried over sodium sulfate then concentrated under reduced pressure to a solid. All of this product was then deprotected in $CHCl_3$ (1 mL) triisopropyl silane (0.15 mL), and TFA (3 mL) for 2.5 h at RT. Excess anhydrous toluene was then added and all volatiles were removed at 50 °C under reduced pressure. The reaction was then diluted into water (8 mL) and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack), the column was then washed with pure water (10 mL), and the product eluted at 20% acetonitrile/water, lyophilized, and found to be >95% pure (by HLPC). The final product was purified further using a C-18 reversed phase HPLC column with an isocratic mixture of 20% acetonitrile (0.1% TFA) in water (0.1% TFA) (3 mL/min) (R_t = 11.5 min) to yield 2.3 mg (29%, 2 steps) of an orange solid. All fluorescein-glycoside conjugates are slightly to moderately hygroscopic, therefore the absorption at 496nm (in aqueous buffer pH 9.0) was used to confirm the yield of the conjugation reaction (taking $\varepsilon_{502\,nm}$ = 77,000 cm^{-1} M^{-1}). ^{1}H-NMR (400 MHz, D_2O) δ 8.17 (d, J = 2.0 Hz, 1H), δ 7.70 (d,d J_1 = 10 Hz, J_2 = 1.6 Hz, 1H), δ 7.15-7.20 (m, 3H), δ 6.97 (d, J = 1.6 Hz, 2H), 6.79-6.80 (m, 2H), δ 5.19 (d, J = 3.2 Hz, 1H), δ 4.91 (d, J = 2.8 Hz, 1H), δ 3.95 (t, J = 6.8 Hz, 1H), δ 3.20-3.60 (m, 23H), δ 2.80 (d,d J_1 = 11.2 Hz, J_2 = 2.4 Hz, 1H),

δ 2.77 (t, J = 5.8 Hz, 2H), δ 2.51-2.60 (m, 4H), δ 1.97-2.07 (m, 2H), δ 1.48-1.53 (m, 2H). MALDI TOF MS calculated for $C_{49}H_{68}N_{16}O_{15}S_2$: 1184.45 found 1185.63 $[M+H]^+$, found 1207.60 $[M+Na]^+$.

[00099] Synthesis and Characterization of Guanidino-Neo-Fluorescein:



Boc_{12}Guanidino$_6$-5"-β-Mercaptoethyl-ether Neomycin B

Guanidino-Neo-Fluorescein

[000100] **Guanidino-Neo-Fluorescein · TFA_6**: Boc_{12} guanidino$_6$-5"-β-mercapto-ethylether neomycin (3 mg, 1.4 μmoles), DMF (0.5 mL), 5-iodo-acetamido-fluorescein (5-IAF) (5 mg, 9.7 μmoles, 1.7 equiv., Molecular Probes), and triethyl amine (20 μL) were stirred at RT in the dark for 2h then diluted into ethyl acetate (150 mL) and washed with 1M Na_2CO_3 (4x50 mL), 0.1M citric acid (2x50 mL), brine (50 mL), dried over sodium sulfate then concentrated under reduced pressure to a solid. All of this product was then deprotected in $CHCl_3$ (3 mL) triisopropyl silane (0.05 mL), and TFA (5 mL) for 3.5 h at RT. Excess anhydrous toluene was then added and all volatiles were removed at 50 °C under reduced pressure. The reaction was then diluted into water (8 mL, 300 mM NaCl) and loaded onto an activated C-18 reversed-phase cartridge (Waters, Sep-pack), the column was then washed with pure water (10 mL), and the product eluted between 5-20% acetonitrile/water (0.001 M HCl), lyophilized, and found to be >85% pure (by HLPC). The product was purified further using a C-18 reversed phase HPLC column with an isocratic mixture of 20% acetonitrile (0.1% TFA) in water (0.1% TFA) (3 mL/min) (R_t = 9.3 min) to yield 1.3 mg (45%, 2 steps) of an orange solid. All fluorescein-glycoside conjugates are slightly to moderately

hygroscopic, therefore the absorption at 496nm (in aqueous buffer pH 9.0) was used to confirm the yield of the conjugation reaction (taking $\varepsilon_{502\ nm}$ = 77,000 cm^{-1} M^{-1}). ^{1}H-NMR (400 MHz, D_2O): δ 8.12 (d, J = 2.0 Hz, 1H), δ 7.72 (d,d J_1 = 8.8 Hz, J_2 = 1.6 Hz, 1H), δ 7.22 (d, J = 8.8 Hz, 1H), δ 7.11 (d, J = 9.2 Hz, 2H), δ 6.92 (d, J = 2.4 Hz, 2H), δ 6.76 (d,d J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), δ 5.69 (d, J = 4.0 Hz, 1H), δ 4.98 (s, 1H), δ 4.87 (s, 1H), δ 4.18-4.24 (m, 2H), δ 3.85-3.88 (m, 3H), δ 3.16-3.62 (m, 18H), δ 2.71-2.78 (m, 4H), δ 2.42-2.52 (m, 4H), δ 2.01 (d t, J_1 = 12.0 Hz, J_2 = 4.4 Hz, 1H), δ 1.48 (q, J = 12.0 Hz, 1H). MALDI TOF MS calculated for $C_{55}H_{79}N_{19}O_{19}S_2$:1373.53, found 1374.72 $[M+H]^+$.

[000101] 10T½ cells, an adherent non-transformed mouse fibroblast cell line, were purchased from ATCC and used before their 20th passage. HeLa cells, a common human cancer cell line, were a gift from Dr. Susan S. Taylor. Both cell lines were cultured in Dulbecco's modified eagle medium containing 10% fetal bovine serum at 37 °C in an 8% CO_2 environment. For each experiment, cells were seeded onto 4 cm tissue culture plates (Nunc) and allowed to grow overnight to ~80% confluency. Compounds 1 – 5 (0.5 – 1 μM) were added to each dish and incubated at 37 °C /8% CO_2 for 0.5 – 1 hr. Cells were then washed once in PBS, trypsinized with 500 μl ATV solution (Gibco) for 3 minutes, pelleted in a fixed angle centrifuge for 5 minutes at 5,000 x G, then brought up in 1 mL PBS. Each sample was quickly (within 5 minutes) analyzed on a FACS VantageSE cell sorter (Becton-Dickinson) using the 488 nm argon/krypton laser line and a 530 nm band pass emission filter. 2,000 – 10,000 cells were counted per sample.

[000102] The uptake of BODIPY-containing glycosides by two different eukaryotic cell lines was also studied using fluorescence microscopy.

[000103] Examples of FACS histograms are presented in Figure 3, and selected microscopy images are shown in Figures 4, 6, 7 and 8. In a typical experiment, cell cultures were treated with 0.5 – 5 μM of each compound for 0.5 – 1 hr, washed twice with buffer, cleaved with trypsin, and quantified for fluorescence at 530 nm.

[000104] Both fluorescent aminoglycosides (1 and 3, see FIG. 3) display poor cellular uptakes (slightly above the autofluorescence of the cell itself) (Table 1).

Table 1. Summary of the mean fluorescence intensities of treated cells according to FACS[a]

Compound	10T1/2[b]	HeLa[c]
None (auto-fluorescence)	~40[d]	830
Tobra-BODIPY (1)	60	1,000
Guanidino-Tobra-BODIPY (2)	240	2,100
Neo-BODIPY (3)	60	~1,400[e]
Guanidino-Neo-BODIPY (4)	430	7,900
BODIPY-Cys $(Arg)_9$ (5)	280	2,000
BODIPY-Cys$(arg)_9$ (5) + 10 μM (6)	110	n.d.[f]
BODIPY-Cys$(arg)_9$ (5) + 50 μM (6)	90	n.d.
BODIPY-Cys$(arg)_9$ (5) + 200 μM (6)	70	n.d.

a) The data between cell types are not directly comparable, as a higher instrumental gain (about 10-fold) was used for the HeLa experiments. (b) Average intensity of 10,000 individual cells treated with 0.5 μM of each compound for 1 hr. (c) Average intensity of 2,000 individual cells treated with 1 μM of each compound for 0.5 hr. Under these conditions a "free" BODIPY dye molecule Tris-BODIPY shows poor uptake into HeLa cells (similar to Tobra-BODIPY). (d) Estimate based upon data set collected at a higher instrument gain. (e) Estimate based upon data set collected at a lower instrument gain. (f) n.d. = not determined.

[000105] Upon guanidinylation, the cellular uptake of tobramycin is enhanced by approximately 10-fold (relative to autofluorescence), and the enhancement for neomycin B is approximately 20-fold (Figure 3A, 3B and Table 1). The type of molecular scaffold used for the display of guanidinium groups has a profound impact on the efficiency of uptake.

[000106] Compared to a common poly-Arg transduction peptide, the guanidinoglycosides show the same, or even better cellular uptake efficiencies. Guanidino-tobra-BODIPY (2) has 4 fewer guanidinium groups as BODIPY-Cys(Arg) (5), but shows approximately the same transport efficiency (Table 1). Importantly, guanidine-neo-BODIPY (4) consistently has a better cellular uptake as compared to the poly-Arg peptide BODIPY-Cys(Arg) (5) (Figure 3C, Table 1). This suggests that the semi-rigid pre-organization of the guanidinium groups on the glycoside core

may better facilitate translocation across the cell membrane. In contrast to the results obtained for a family of poly-Arg peptoids, the flexible amphipathic properties usually provided by the methylene chains of poly(Arg) residues do not appear essential for membrane transport of guanidinoglycosides. To address the possibility that guanidine-neomycin B enters cells through a different mechanism than poly-Arg, a competition experiment was conducted between BODIPY-Cys(Arg) (5) and the unlabeled guanidine-neomycin B (6, see FIG. 2). FACS analysis shows that guanidine-neomycin B (6, see FIG. 2) effectively inhibits the transport of BODIPY-Cys(Arg) into cells (Figure 3D and Table 1), suggesting a common pathway responsible for the uptake of both compounds.

[000107] Microscopy experiments have been conducted using both fluorescein-labeled and BODIPY-labeled guanidinoglycosides. The relative intensities of individual cells, following treatment with either fluorescent aminoglycosides or guanidinoglycosides are consistent with the trends from FACS experiments. Optical cross sectioning using scanning confocal fluorescence microscopy indicates that guanidinoglycosides are found inside of living cells (Figure 8). Interestingly, two distinct types of cellular localization of guanidino-neo-BODIPY are observed (Figure 8). Approximately half of the cells exhibit a highly diffuse, cytoplasmic and nuclear distribution (Figure 6), while the other half exhibit more localized nucleolar staining, similar to that reported for poly-Arg peptides (Figure 7). Similar results were observed with fluorescein-labeled conjugates, as well as 10T½ cells (Figure 8). Taken together, this suggests that the relative uptake efficiencies and cellular localization of these compounds are not highly dependent on cell type or dye molecules used.

[000108] In summary, unlike aminoglycosides, guanidinoglycosides exhibit highly efficient uptake by eukaryotic cell cultures via a similar mechanism as a poly-arginine peptide.

EXAMPLE 2 HIV INHIBITION

[000109] Evaluation of HIV-1 Inhibitory Activity

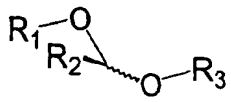
[000110] The analytically pure and fully characterized conjugates will be tested for their ability to inhibit HIV replication in HIV-1 infected CD44 HeLa cells by following their inhibition of plaque formation. The decrease in viral load in infected human peripheral blood monocytes (determined by standard p24 ELISA) will also be carried.

[000111] The performance of the AZT-guanidino-neomycin B conjugate, for example, will be evaluated against the individual components. These controls include: 1) AZT alone, 2) linker alone, 3) guanidino-neomycin B alone, 4) AZT + guanidino-neomycin B, 5) AZT-monophosphate alone, and 6) AZT-monophosphate + guanidine-neomycin B. In each case dose-dependent curves will be generated in triplicates.

[000112] Although the invention has been described with reference to the above examples, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

WHAT IS CLAIMED IS:

1. A method of increasing the cellular uptake of a compound comprising conjugating the compound with a molecule having at least one cyclic acetal, wherein the at least one cyclic acetal has the generic formula:



wherein R1, R2, and/or R3 groups comprise two or more 5- or 6-membered rings which are linked together by at least one acetal-type functional groups and wherein R1-R2, and R3 are the carbon atoms of two separate ring systems.

2. The method of claim 1, wherein the at least one cyclic acetal is guanidinylated.

3. The method of claim 1, wherein the at least one cyclic acetal is a glycoside.

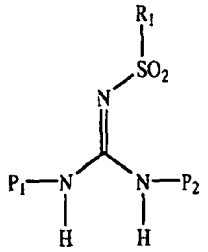
4. The method of claim 3, wherein the glycoside is an aminoglycoside.

5. The method of claim 3 or 4, wherein at least one primary or secondary alcohol or at least one primary or secondary amine of the glycoside is reacted with a guanidinylating reagent to produce a guanidinoglycoside.

6. The method of claim 1, wherein the conjugate is covalently bonded.

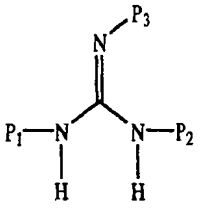
7. The method of claim 5, wherein the guanidinylating reagent has the general formula:

(I)



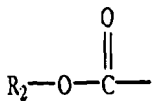
for primary and secondary amines or

(III)



for primary and secondary alcohols,

wherein for (I), P_1 and P_2 are urethane protecting groups and R_1 is trifluoromethane, and wherein for (III), P_1, P_2 and P_3 are the same or different urethane protecting groups, each urethane protecting group having the general structure:



wherein R_2 is a substituted or unsubstituted alkyl or aryl group or heterocyclic group.

8. The method of claim 7, wherein the molecule is selected from the group consisting of amikacin, gentamicin, kanamycin, neomycin, netilmicin, O-2,6-Diamino-2,6-dideoxy-beta-L-idopyranosyl-(1 to 3)-O-beta-D-ribofuranosyl-(1 to 5)-O-[2-amino-2-deoxy-alpha-D-glucopyranosyl-(1 to 4)]-2-deoxystreptamine, streptomycin, tobramycin, ouabain, deslanoside, digoxin, digitoxin, lantoside and strophanthin.

9. The method of claim 1, wherein the compound is selected from the group consisting of a nucleic acid, nucleoside, protein, peptide, amino acid residue, lipid, carbohydrate, synthetic organic compound, metal, vitamin, small molecule, dye, isotope, antibody, toxin and ligand.

10. The method of claim 9, wherein the compound comprises a nucleoside, wherein the nucleoside is a reverse transcriptase inhibitor.

11. The method of claim 10, wherein the reverse transcriptase inhibitor is selected from the group consisting of 3'-azido-3'-deoxythymidine, 2',3'-dideoxyinosine and 2',3'-dideoxycytidine.

12. The method of claim 11, wherein the reverse transcriptase inhibitor is conjugated to an aminoglycoside.

13. The method of claim 12, wherein the aminoglycoside is selected from the group consisting of amikacin, gentamicin, kanamycin, neomycin, netilmicin, O-2,6-Diamino-2,6-dideoxy-beta-L-idopyranosyl-(1 to 3)-O-beta-D-ribofuranosyl-(1 to 5)-O-[2-amino-2-deoxy-alpha-D-glucopyranosyl-(1 to 4)]-2-deoxystreptamine, streptomycin and tobramycin.

ABSTRACT OF THE INVENTION

COMPOSITIONS AND METHODS FOR USE OF GUANIDINIUM DERIVATIVES OF GLYCOSIDES FOR IMPROVED CELLULAR TRANSPORT

Provided are reagents and methods useful for the synthesis of conjugates comprising guanidinylated cyclic acetals. Also provided are methods for increasing the cellular uptake of various therapeutic compounds and treatment modalities using these conjugates.

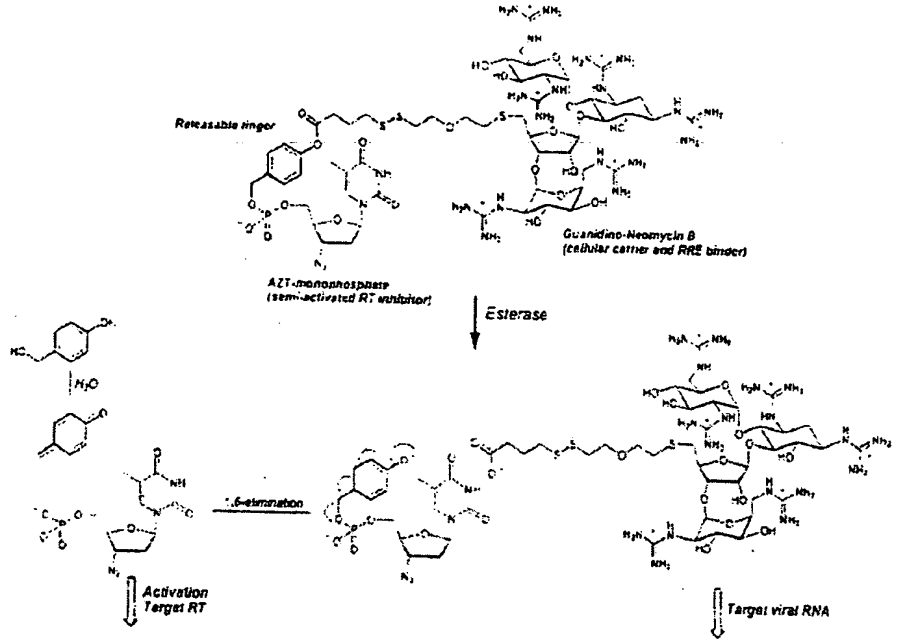

Releasable linker
Guanidino-Neomycin B
(cellular carrier and RRE binder)
AZT-monophosphate
(semi-activated RT inhibitor)
Esterase
H2O
β-elimination
Activation
Target RT
Target viral RNA

Figure 1

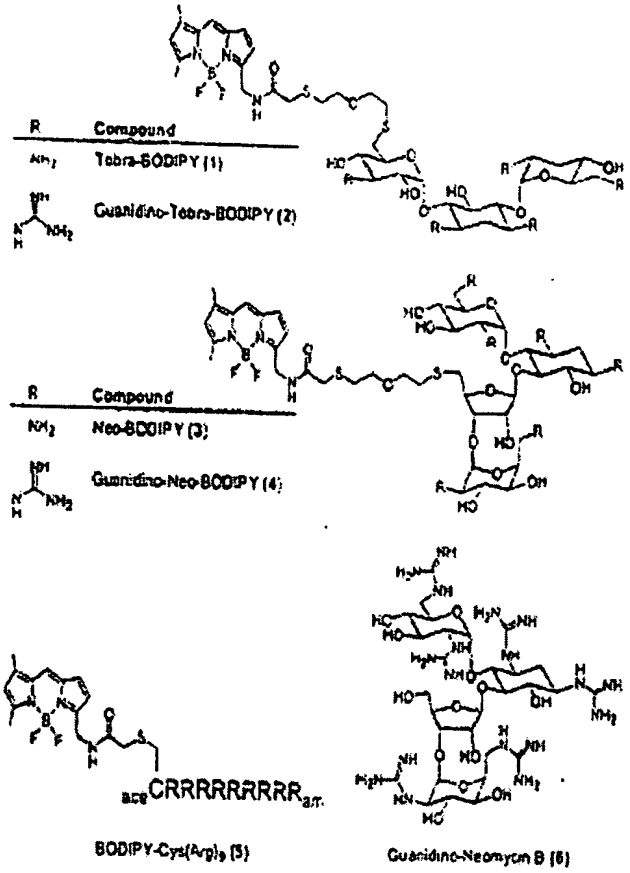

R
Compound
Tobra-BODIPY (1)
Guanidino-Tobra-BODIPY (2)
R
Compound
Neo-BODIPY (3)
Guanidino-Neo-BODIPY (4)
BODIPY-Cys(Arg)9 (5)
Guanidino-Neomycin B (6)

Figure 2

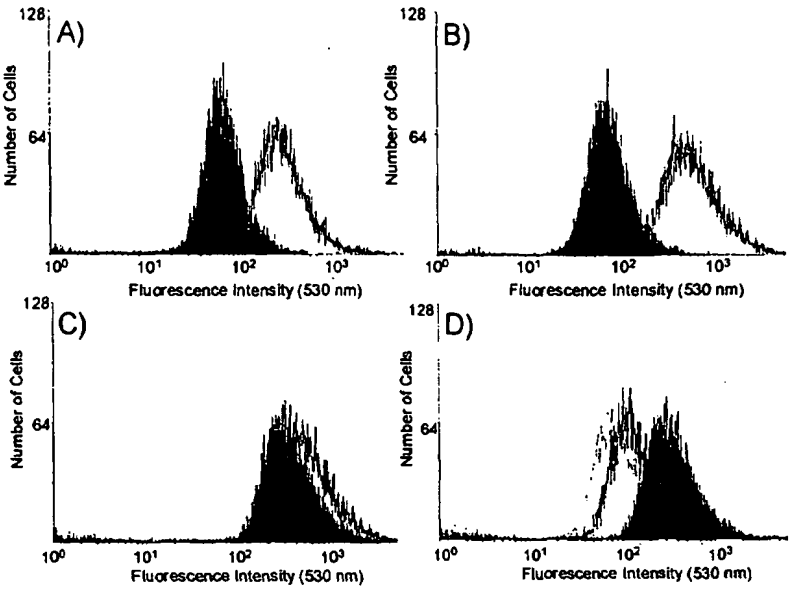

A)
B)
C)
D)
128
64
Number of Cells
10^0
10^1
10^2
10^3
Fluorescence Intensity (530 nm)

Figure 3

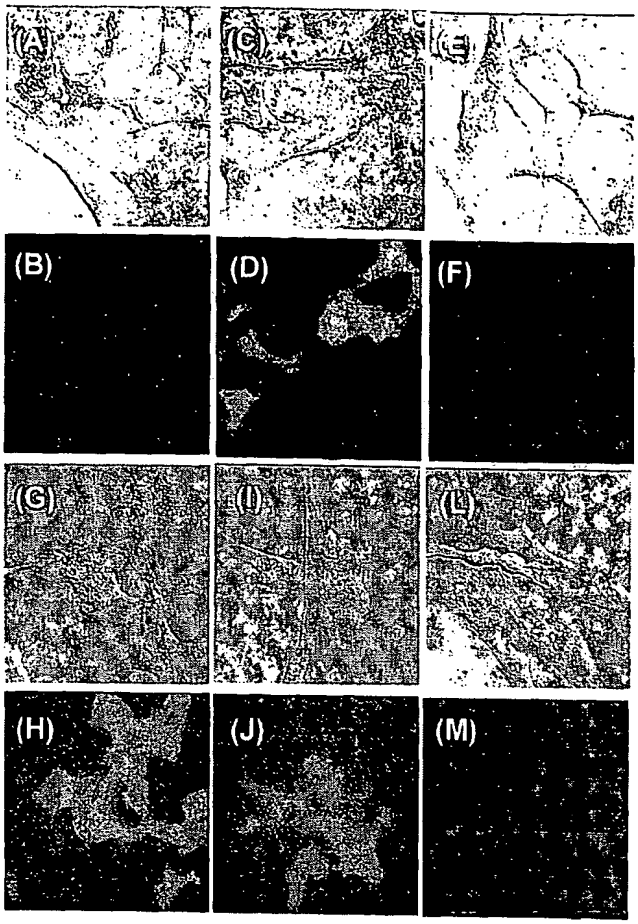
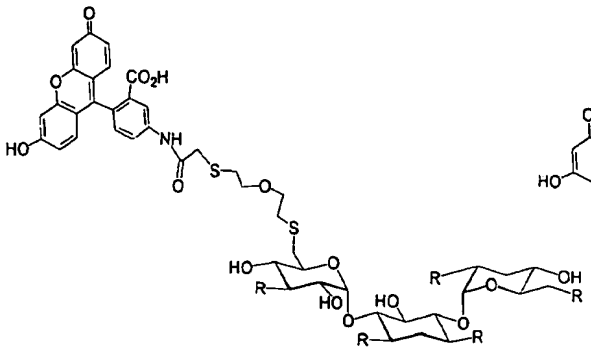
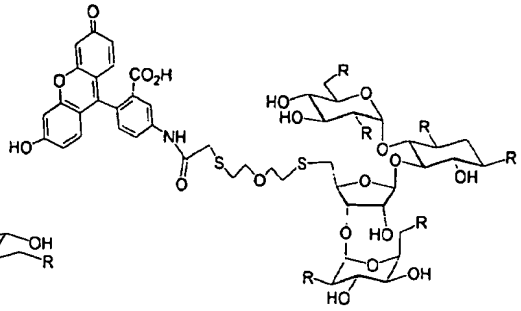


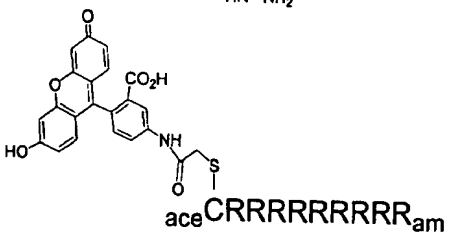
Figure 4



R	Compound
$-NH_2$	Amino-Tobra-Fluorescein
	Guanidino-Tobra-Fluorescein

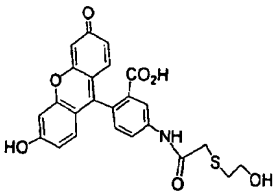


R	Compound
	Guanidino-Neo-Fluorescein



aceCRRRRRRRRR$_{am}$

Fluorescein–Cys(Arg)$_9$



βMe-Fluorescein

Figure 5

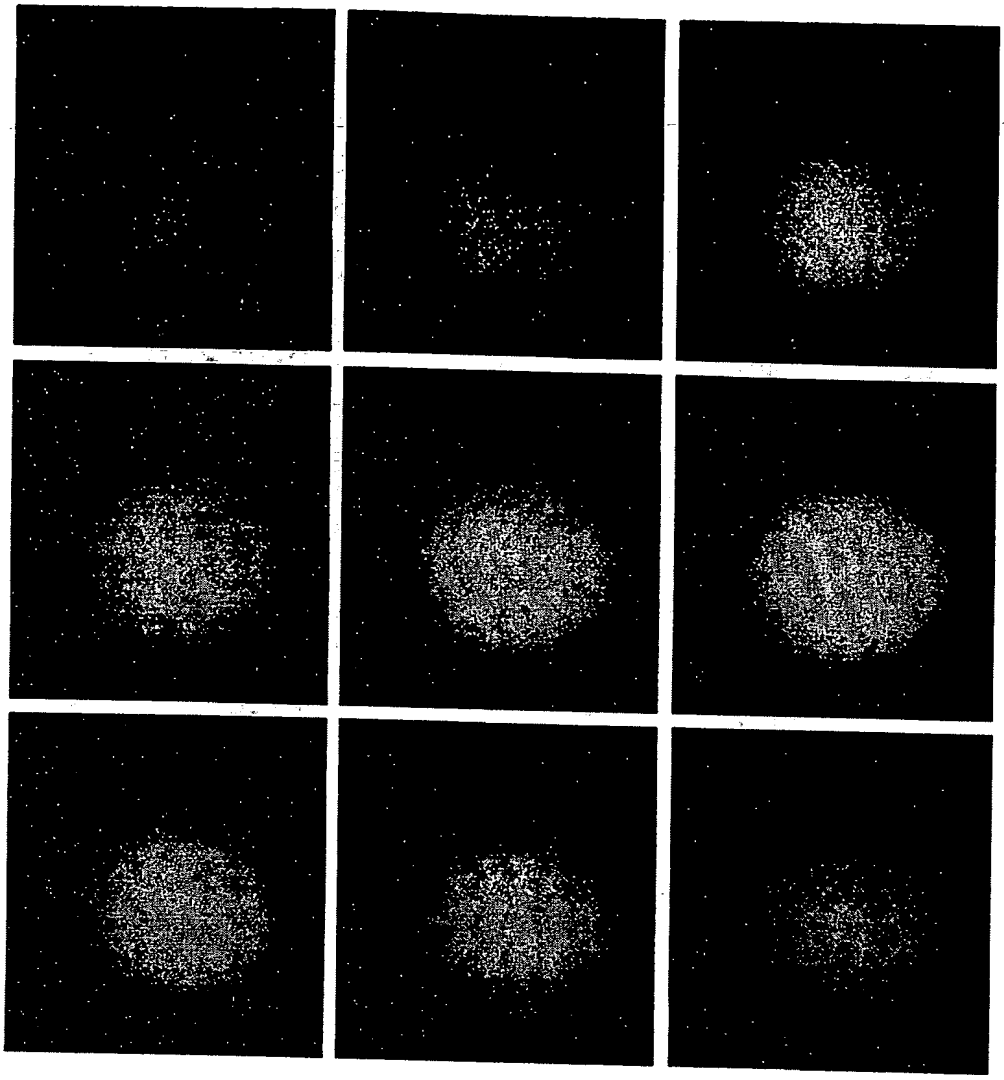


Figure 6

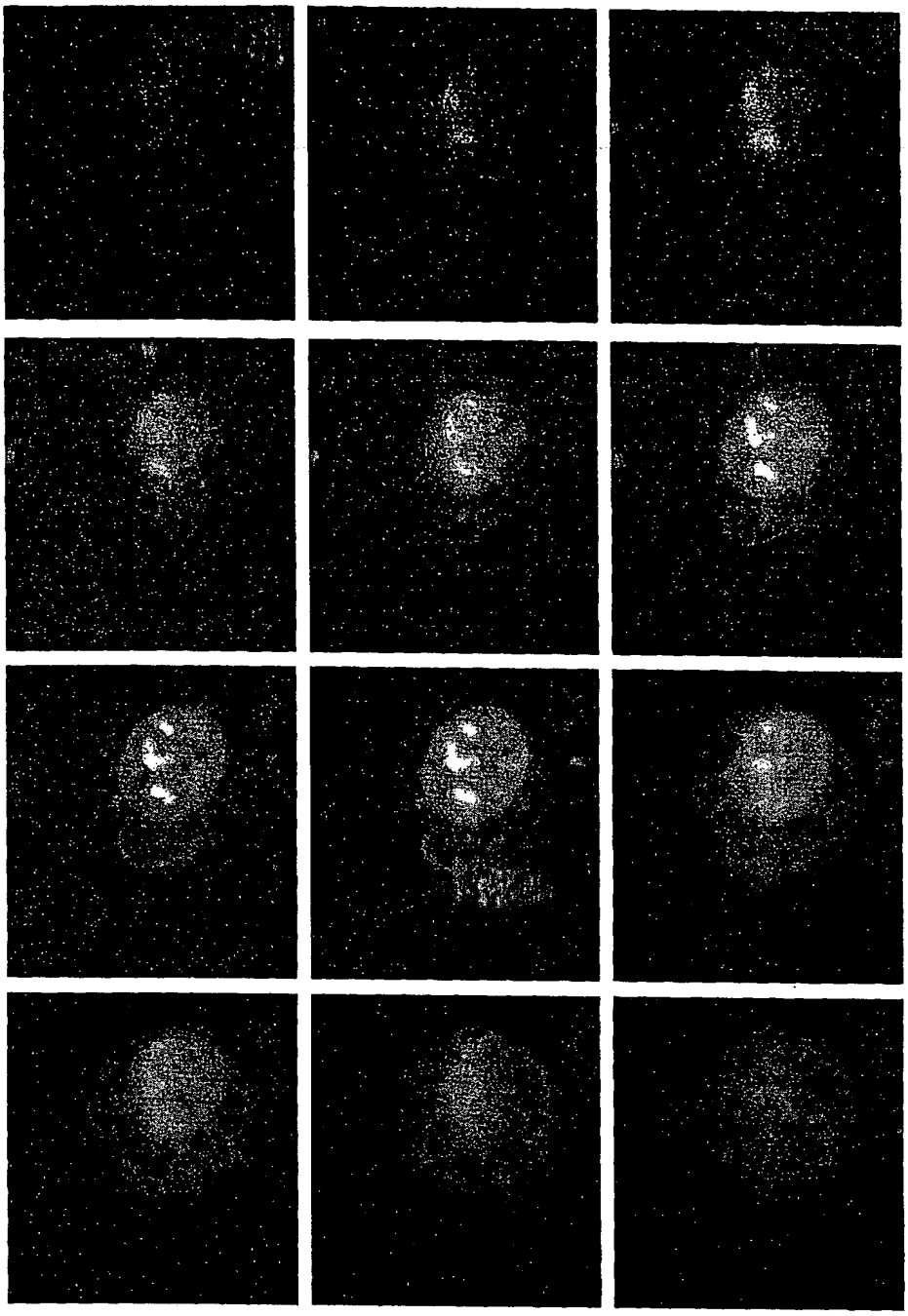


Figure 7

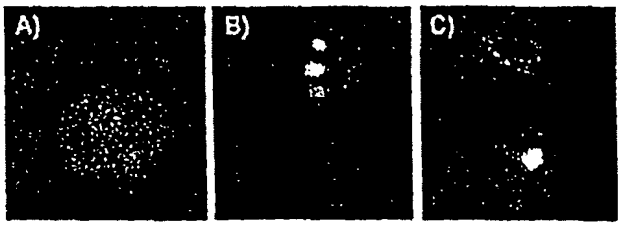


Figure 8

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